

REVISED VERSION

(19) World Intellectual Property Organization International Bureau



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17 April 2003 (17.04.2003)

PCT

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PCT/GB2002/004619

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(26) Publication Language: English

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0124317.9 10 October 2001 (10.10.2001) GB

(71) Applicant (for all designated States except US): CELL-TECH R & D LIMITED [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(72) Inventors; and

(75) Inventors/Applicants (for US only): POPPLEWELL,

Andrew, George [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). TICKLE, Simon, Peter [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). ZINKEWICH-PEOTTI, Karen [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). MORRISON, Robert, Kendall [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

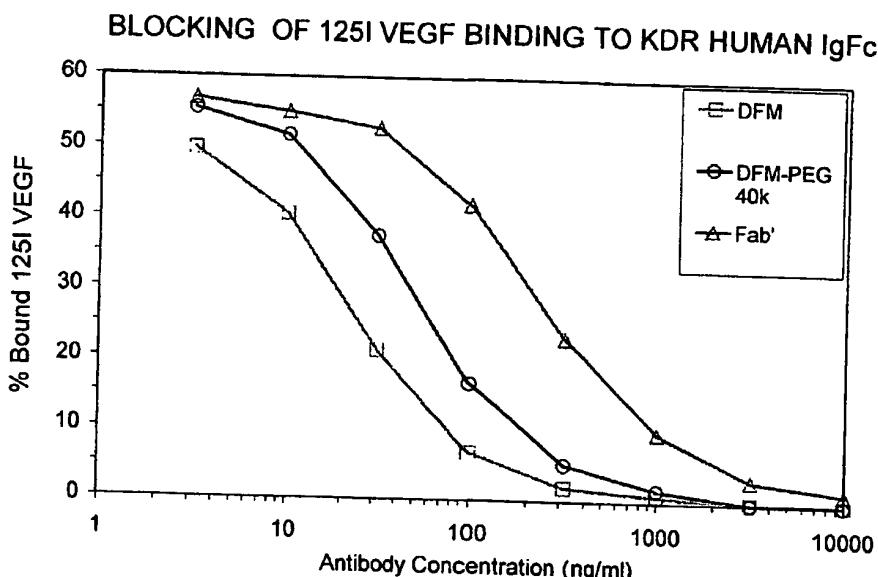
(74) Agents: MERCER, Christopher, Paul et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

[Continued on next page]

(54) Title: ANTIBODIES AGAINST KDR, THEIR PRODUCTION AND USES

Radioimmunoassay Results



WO 2003/031475 A3

(57) Abstract: There are disclosed antibody molecules containing at least one CDR derived from a mouse monoclonal antibody having specificity for human KDR. There is also disclosed a CDR grafted antibody wherein at least one of the CDRs is a hybrid CDR. Further disclosed are DNA sequences encoding the chains of the antibody molecules, vectors, transformed host cells and uses of the antibody molecules in the treatment of diseases in which VEGF and/or KDR are implicated.



(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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25 March 2004

Date of publication of the revised international search report:

14 October 2004

(15) Information about Correction:

see PCT Gazette No. 42/2004 of 14 October 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/04619

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C07K16/28	C07K16/46	A61K39/395	A61K47/48	C12N15/13
	C12N15/70	C12N1/21	G01N33/577	A61P17/06	A61P37/00
	A61P19/02	A61P35/04			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, MEDLINE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WITTE L ET AL: "MONOClonal ANTIBODIES TARGETING THE VEGF RECEPTOR-2 (FLK1/KDR) AS AN ANTI-ANGIOGENIC THERAPEUTIC STRATEGY"</p> <p>CANCER AND METASTASIS REVIEWS, KLUWER ACADEMIC PUBLISHERS, DORDRECHT, NL, vol. 17, no. 2, 1998, pages 155-161, XP000940443</p> <p>ISSN: 0167-7659</p> <p>abstract</p> <p>page 155, left-hand column, paragraph 1</p> <p>page 160, right-hand column, paragraph 1</p> <p>the whole document</p> <p>-----</p> <p style="text-align: center;">-/--</p>	1-21, 28-40
Y		22-27

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the International filing date but later than the priority date claimed

- *T* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the International search

15 September 2003

Date of mailing of the International search report

09.01.04

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Irion, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/04619

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HICKLIN DANIEL J ET AL: "Monoclonal antibody strategies to block angiogenesis." DRUG DISCOVERY TODAY, vol. 6, no. 10, 2001, pages 517-528, XP002254076 ISSN: 1359-6446 page 520, right-hand column, paragraph 5 - page 522, left-hand column, paragraph 2 the whole document -----	1-21, 28-40
Y		22-27
X	ZHU Z ET AL: "INHIBITION OF VASCULAR ENDOTHELIAL GROWTH FACTOR INDUCED MITOGENESIS OF HUMAN ENDOTHELIAL CELLS BY A CHIMERIC ANTI-KINASE INSERT DOMAIN-CONTAINING RECEPTOR ANTIBODY" CANCER LETTERS, NEW YORK, NY, US, vol. 136, no. 2, 1 March 1999 (1999-03-01), pages 203-213, XP000907699 ISSN: 0304-3835 abstract the whole document -----	1-21, 28-40
Y		22-27
X	WO 00/44777 A (IMCLONE SYSTEMS INC ;WITTE LARRY (US); ZHU ZHENPING (US)) 3 August 2000 (2000-08-03) page 1, line 8 - line 9 the whole document ----- page 1, line 26 page 5, line 4 - line 9 page 5, line 21 page 9, line 20 - line 27 page 9, line 28 - page 10, line 7 claims 48,49 -----	1-21, 28-40
Y		22-27
X	WO 98/11223 A (MARTINY BARON GEORG ;SCHERING AG (DE); MENRAD ANDREAS (DE); TOTZKE) 19 March 1998 (1998-03-19) abstract page 2, line 13 - line 14 page 3, line 10 - line 13 the whole document -----	1-21, 28-40
A		22-27
X	EP 1 086 705 A (KYOWA HAKKO KOGYO KK) 28 March 2001 (2001-03-28) page 4, line 4 - line 5 page 4, line 53 - line 57 page 5, line 43 - line 48 the whole document -----	1-21, 28-40
A		22-27
Y	US 6 133 426 A (LEONG STEVEN R ET AL) 17 October 2000 (2000-10-17) column 121 - column 122 -----	22-27
		-/-

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/04619

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>HURWITZ ESTHER ET AL: "Inhibition of tumor growth by poly(ethylene glycol) derivatives of anti-ErbB2 antibodies." CANCER IMMUNOLOGY IMMUNOTHERAPY, vol. 49, no. 4-5, July 2000 (2000-07), pages 226-234, XP002254077 ISSN: 0340-7004 abstract</p> <p>-----</p>	22-27
A	<p>DECKERT P MARKUS ET AL: "Pharmacokinetics and microdistribution of polyethylene glycol-modified humanized A33 antibody targeting colon cancer xenografts." INTERNATIONAL JOURNAL OF CANCER, vol. 87, no. 3, 2000, pages 382-390, XP002254078 ISSN: 0020-7136 abstract</p> <p>-----</p>	22-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/04619

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple Inventions in this International application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

see PCT/ISA/210 annex

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-40

Group I: an antibody molecule having specificity for human KDR, the DNA sequence which encodes said antibody molecule, a vector containing the DNA sequence, a host cell transformed with said vector, a process for the production of the antibody molecule, and a therapeutic composition comprising said antibody molecule and its use.

2. claim: 41

Group II: a vector designated pTTOD(CDP791) as shown in Figure 8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 02/04619

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0044777	A 03-08-2000	AU 3475100	A	18-08-2000
		CA 2361553	A1	03-08-2000
		CN 1345334	T	17-04-2002
		EP 1151002	A1	07-11-2001
		JP 2002536968	T	05-11-2002
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		AU 730287	B2	01-03-2001
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US 6133426	A 17-10-2000	US 6025158	A	15-02-2000
		US 6458355	B1	01-10-2002
		US 6468532	B1	22-10-2002
		US 2003021790	A1	30-01-2003

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(51) International Patent Classification⁷: **C07K 16/28**, 16/46, A61K 39/395, 47/48, C12N 15/13, 15/70, 1/21, G01N 33/577, A61P 17/06, 37/00, 19/02, 35/04

Berkshire SL1 3WE (GB). TICKLE, Simon, Peter [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). ZINKEWICH-PEOTTI, Karen [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). MORRISON, Robert, Kendall [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(21) International Application Number:
PCT/GB2002/004619

(74) Agents: MERCER, Christopher, Paul et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

(22) International Filing Date: 10 October 2002 (10.10.2002)

(25) Filing Language: English

(26) Publication Language: English

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(30) Priority Data:
0124317.9 10 October 2001 (10.10.2001) GB

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),

(71) Applicant (*for all designated States except US*): **CELL-TECH R & D LIMITED [GB/GB]**; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

(72) Inventors; and

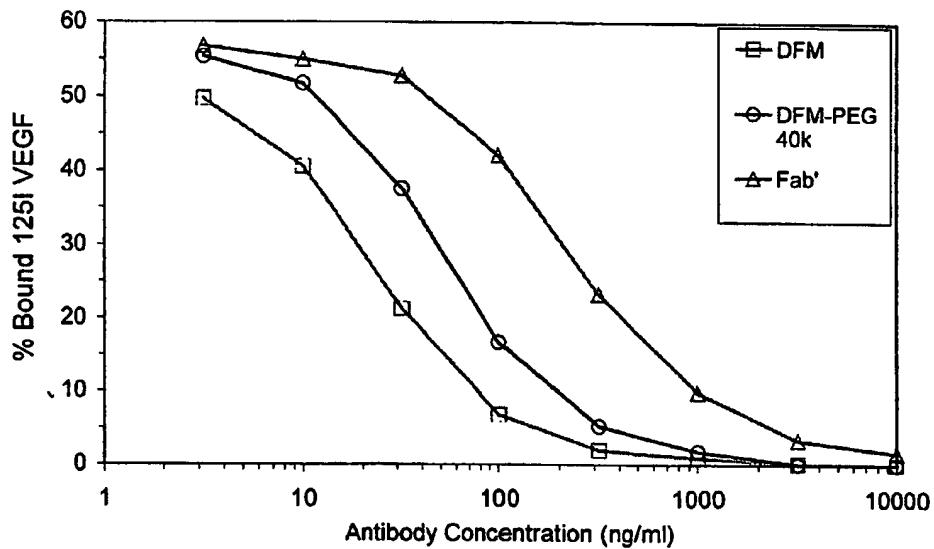
(75) Inventors/Applicants (*for US only*): **POPPLEWELL, Andrew, George [GB/GB]**; 208 Bath Road, Slough,

[Continued on next page]

(54) Title: **BIOLOGICAL PRODUCTS**

Radioimmunoassay Results

BLOCKING OF 125 I VEGF BINDING TO KDR HUMAN IgFc



(57) **Abstract:** There are disclosed antibody molecules containing at least one CDR derived from a mouse monoclonal antibody having specificity for human KDR. There is also disclosed a CDR grafted antibody wherein at least one of the CDRs is a hybrid CDR. Further disclosed are DNA sequences encoding the chains of the antibody molecules, vectors, transformed host cells and uses of the antibody molecules in the treatment of diseases in which VEGF and/or KDR are implicated.

WO 2003/031475 A3



European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(88) Date of publication of the international search report:
25 March 2004

Published:

— with international search report

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INTERNATIONAL SEARCH REPORT

Inten I Application No
PCT/GB 02/04619

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C07K16/28	C07K16/46	A61K39/395	A61K47/48	C12N15/13
	C12N15/70	C12N1/21	G01N33/577	A61P17/06	A61P37/00
	A61P19/02	A61P35/04			

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data, PAJ, EMBASE, MEDLINE, Sequence Search

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Y		22-27

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Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
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- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

15 September 2003

Date of mailing of the international search report

19.01.04

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl.
Fax: (+31-70) 340-3016

Authorized officer

Irion, A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 02/04619

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A		22-27
Y	US 6 133 426 A (LEONG STEVEN R ET AL) 17 October 2000 (2000-10-17) column 121 - column 122 -----	22-27
		-/-

INTERNATIONAL SEARCH REPORT

Int'l Application No
PCT/GB 02/04619

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HURWITZ ESTHER ET AL: "Inhibition of tumor growth by poly(ethylene glycol) derivatives of anti-ErbB2 antibodies." CANCER IMMUNOLOGY IMMUNOTHERAPY, vol. 49, no. 4-5, July 2000 (2000-07), pages 226-234, XP002254077 ISSN: 0340-7004 abstract -----	22-27
A	DECKERT P MARKUS ET AL: "Pharmacokinetics and microdistribution of polyethylene glycol-modified humanized A33 antibody targeting colon cancer xenografts." INTERNATIONAL JOURNAL OF CANCER, vol. 87, no. 3, 2000, pages 382-390, XP002254078 ISSN: 0020-7136 abstract -----	22-27

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/04619

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

see additional sheet

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the Invention first mentioned in the claims; it is covered by claims Nos.:

see PCT/ISA/210 annex

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/SA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-40

Group I: an antibody molecule having specificity for human KDR, the DNA sequence which encodes said antibody molecule, a vector containing the DNA sequence, a host cell transformed with said vector, a process for the production of the antibody molecule, and a therapeutic composition comprising said antibody molecule and its use.

2. claim: 41

Group II: a vector designated pTTOD(CDP791) as shown in Figure 8

INTERNATIONAL SEARCH REPORT

 Inte
Application No
PCT/GB 02/04619

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO 0044777	A 03-08-2000	AU 3475100	A	18-08-2000
		CA 2361553	A1	03-08-2000
		CN 1345334	T	17-04-2002
		EP 1151002	A1	07-11-2001
		JP 2002536968	T	05-11-2002
		WO 0044777	A1	03-08-2000
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		WO 9811223	A1	19-03-1998
		EP 0925359	A1	30-06-1999
		HU 9904052	A2	28-03-2000
		JP 2001501920	T	13-02-2001
		NO 991162	A	06-05-1999
		PL 332034	A1	16-08-1999
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		ZA 9708204	A	03-03-1998
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EP 1086705	A 28-03-2001	AU 3850299	A	06-12-1999
		CA 2328893	A1	25-11-1999
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US 6133426	A 17-10-2000	US 6025158	A	15-02-2000
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		US 6468532	B1	22-10-2002
		US 2003021790	A1	30-01-2003

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
17 April 2003 (17.04.2003)

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(GB). ZINKEWICH-PEOTTI, Karen [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB). MORRISON, Robert, Kendall [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

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(74) Agents: MERCER, Christopher, Paul et al.; Carpmaels & Ransford, 43 Bloomsbury Square, London WC1A 2RA (GB).

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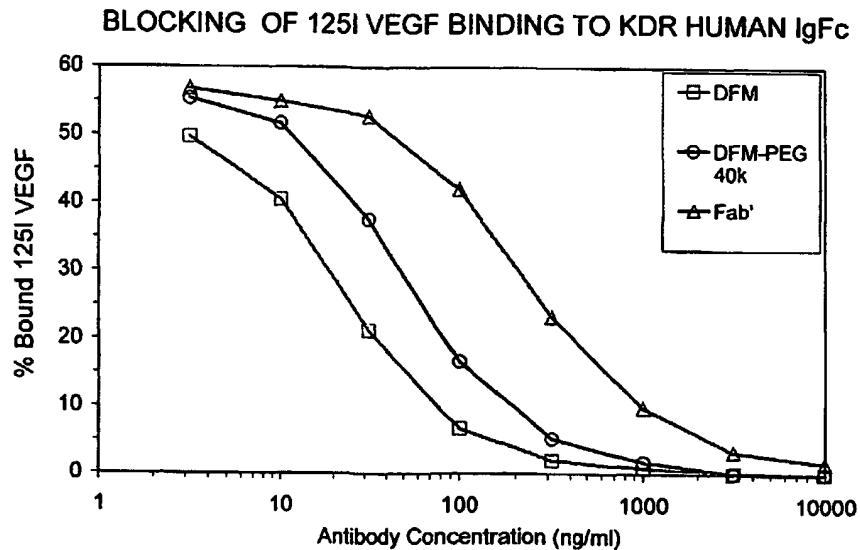
(71) Applicant (for all designated States except US): CELL-TECH R & D LIMITED [GB/GB]; 208 Bath Road, Slough, Berkshire SL1 3WE (GB).

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(54) Title: BIOLOGICAL PRODUCTS

Radioimmunoassay Results



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(57) Abstract: There are disclosed antibody molecules containing at least one CDR derived from a mouse monoclonal antibody having specificity for human KDR. There is also disclosed a CDR grafted antibody wherein at least one of the CDRs is a hybrid CDR. Further disclosed are DNA sequences encoding the chains of the antibody molecules, vectors, transformed host cells and uses of the antibody molecules in the treatment of diseases in which VEGF and/or KDR are implicated.



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Biological Products

The present invention relates to an antibody molecule having specificity for antigenic determinants of human kinase insert domain-containing receptor (KDR). The 5 antibody molecule binds KDR with greater affinity than human vascular endothelial growth factor (VEGF) and prevents the interaction between VEGF and KDR. The present invention also relates to the therapeutic uses of the antibody molecule and methods for producing the antibody molecule.

This invention relates to antibody molecules. In an antibody molecule, there are two heavy 10 chains and two light chains. Each heavy chain and each light chain has at its N-terminal end a variable domain. Each variable domain is composed of four framework regions (FRs) alternating with three complementarity determining regions (CDRs). The CDRs determine the antigen binding specificity of antibodies and are relatively short peptide sequences carried on the framework regions of the variable domains. The residues in the 15 variable domains are conventionally numbered according to a system devised by Kabat et al. This system is set forth in Kabat et al., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA (hereafter "Kabat et al. (supra)"). This numbering system is used in the present specification except where otherwise indicated.

20 The Kabat residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering, corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. The correct Kabat numbering of residues may be 25 determined for a given antibody by alignment of residues of homology in the sequence of the antibody with a "standard" Kabat numbered sequence.

The CDRs of the heavy chain variable domain are located at residues 31-35 (CDRH1), residues 50-65 (CDRH2) and residues 95-102 (CDRH3) according to the Kabat numbering.

30 The CDRs of the light chain variable domain are located at residues 24-34 (CDRL1), residues 50-56 (CDRL2) and residues 89-97 (CDRL3) according to the Kabat numbering.

Construction of CDR-grafted antibodies is described in European Patent Application EP-A-0239400, which discloses a process in which the CDRs of a mouse monoclonal antibody (Mab) are grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long 5 oligonucleotides.

The earliest work on humanising Mabs by CDR-grafting was carried out on Mabs recognising synthetic antigens, such as NP. However, examples in which a mouse Mab recognising lysozyme and a rat Mab recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen et al. (Science, 239, 1534-10 1536, 1988) and Riechmann et al. (Nature, 332, 323-324, 1988), respectively.

Riechmann et al. found that the transfer of the CDRs alone (as defined by Kabat (Kabat et al. (supra) and Wu et al., J. Exp. Med., 132, 211-250, 1970)) was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. It was found that a number of framework residues have to be altered so that they correspond to those of the 15 donor framework region. Proposed criteria for selecting which framework residues need to be altered are described in International Patent Application No. WO 90/07861.

A number of reviews discussing CDR-grafted antibodies have been published, including Vaughan et al. (Nature Biotechnology, 16, 535-539, 1998).

VEGF is a homodimeric glycoprotein of two 23kD subunits with structural 20 similarity to PDGF. It has an important developmental role in vasculogenesis, the establishment of a system of new blood vessels, and is involved in angiogenesis, the formation of new vessels from pre-existing ones. Angiogenesis involves the proliferation, migration and tissue infiltration of capillary endothelial cells from pre-existing blood vessels. As well as playing an important role in normal physiological processes, such as 25 embryonic development, follicular growth (including corpus luteum formation) and wound healing, angiogenesis occurs in a number of pathological conditions including inflammation, psoriasis, rheumatoid arthritis and tumour growth and metastasis (Folkman, J and Klagsbrun, M., Science, 235:442-447, 1987). For example, it is widely believed that 30 tumours are incapable of growing beyond a certain size unless they are provided with a dedicated blood supply via angiogenesis.

VEGF is distinct from other factors implicated as possible regulators of angiogenesis *in vivo* in that it is an endothelial cell-specific angiogenesis inducer.

Five different monomeric isoforms of VEGF exist, resulting from alternative splicing of mRNA. The isoforms include two membrane bound forms (VEGF₂₀₆ and VEGF₁₈₉) and three soluble forms (VEGF₁₆₅, VEGF₁₂₁ and VEGF₁₄₅). In all tissues except human placenta, VEGF₁₆₅ is the most abundant isoform.

5 The effects of VEGF are mediated through its interaction with two high affinity tyrosine kinase receptors, fms-like tyrosine kinase receptor (FLT-1 or VEGFR-1, Shibuya M. et al., *Oncogene*, 5, 519-524, 1990) and KDR (or VEGFR-2, Terman et al., *Oncogene*, 6, 1677-1683, 1991). Both KDR and FLT-1 are membrane-spanning receptors that each contain seven immunoglobulin-like domains in the extracellular ligand-binding region, an 10 intracellular tyrosine kinase domain and a transmembrane domain. The transmembrane domain serves to anchor the receptor in the cell membrane of the cells in which it is expressed.

There are several reports of the over-expression of both VEGF and its receptors within tumours, both at the RNA and protein levels (Dvorak et al., *Curr. Top. Microbiol. 15 Imunol.*, 237, 97, 1999). VEGF expression is upregulated in response to hypoxia, which frequently occurs within tumours, and increased concentration of ligand induces the expression of its receptors. Examples of studies showing increased KDR expression in human tumours include: breast cancer (Brown et al., *Hum. Pathol.*, 26, 86, 1995); colon cancer (Takahashi et al., *Cancer Res.*, 55, 3964, 1995); renal cancer (Takahashi et al, 20 *BBRC* 257, 855, 1999) and adenocarcinoma of the gastro-intestinal tract (Brown et al., *Cancer Res.*, 53, 4727, 1993). In a more recent study using an antibody specifically recognising VEGF bound to KDR, upregulation of the VEGF/KDR angiogenic pathway in non-small cell lung cancer was observed (Koukourakis et al., *Cancer Res.*, 60, 3088, 2000).

A number of pieces of experimental evidence demonstrate the causal link between 25 VEGF activity and tumour angiogenesis *in vivo*. Kim et al. injected an anti-VEGF neutralising Mab into tumour-bearing nude mice and showed suppressed tumour growth (*Nature* 362, 841, 1993). Retroviral expression of a dominant negative mouse KDR (FLK-1) also inhibited tumour growth in mice (Millauer et al., *Nature*, 367, 576, 1993). Similarly, VEGF antisense (Cheng et al., *PNAS*, 93, 8502, 1996), anti-FLK-1 antibodies 30 (Witte et al., *Cancer Metast. Rev.*, 17, 155, 1998) and expression of soluble FLT-1 (Goldman et al., *PNAS*, 95, 8795, 1998) all inhibited tumour growth in mouse models.

Several pieces of experimental evidence suggest the biological effects of VEGF relating to angiogenesis are mediated predominantly through the KDR receptor (for review see Larrivee and Karsan, *Int. J. Mol. Med.*, 5, 447, 2000).

The VEGF-mediated activation of KDR alone (in cell lines expressing one VEGFR-type only) was shown to be sufficient to cause cell proliferation and migration (Waltenburger et al., *J. Biol. Chem.*, 269, 26988, 1994). Conversely, when FLT-1 alone is activated, cell proliferation is not seen and cell migration is inconsistently observed.

Experiments utilising receptor-selective VEGF mutants have shown that KDR ligation activates mitogen-activated protein kinase (MAPK) giving rise to proliferation, migration and vascular permeability (Keyt et al., *J. Biol. Chem.*, 271, 5638, 1996). The FLT-1 selective mutant was inactive in these assays.

An anti-VEGF Mab blocking the interaction with KDR but not FLT-1 was able to inhibit VEGF-induced vascular permeability, whereas a non-blocking anti-VEGF antibody had no effect (Brekken et al., *Cancer Res.*, 60, 5117, 2000).

The production of Mabs against the murine VEGF receptor, FLK-1, by hybridoma technology has been described (WO 94/11499). These were demonstrated to inhibit FLK-1 receptor activation by blocking the interaction of VEGF with the receptor. This inhibition of receptor activation was effective in inhibiting VEGF-induced angiogenesis in certain models. In addition, this anti-FLK-1 antibody has proven effective in treating several mouse xenograft tumours. However, not all antibodies that bind FLK-1 will bind KDR with sufficient affinity for therapeutic efficacy.

VEGF-KDR binding also inhibits apoptosis of newly formed blood vessels via the KDR-mediated activation of the PI3-kinase-Akt kinase signalling pathways (Akt kinase is a well-known downstream kinase of the PI3-kinase pathway involved in cell survival, Gerber et al., *J. Biol. Chem.*, 273, 30336, 1998). Animal models also demonstrated the effectiveness of blockade of this anti-apoptotic response through blocking the VEGF-KDR interaction.

It is currently believed that KDR is the most important receptor in mediating the effects of VEGF and its role in promoting angiogenesis and new vessel survival appears universally acknowledged.

Therefore, an antibody molecule able to bind KDR and block its activation by VEGF may be of therapeutic benefit in the treatment of pathologies in which VEGF and/or KDR are implicated. For example, cases of inflammation, psoriasis, rheumatoid arthritis

and tumour growth. There are also strong arguments that this may be best achieved through blocking its interaction with the KDR receptor. There is a need for such an antibody molecule which can be used repeatedly and produced easily and efficiently. There is also a need for an antibody molecule that has high affinity for KDR and low 5 immunogenicity in humans.

In a first aspect, the present invention provides an antibody molecule having specificity for KDR, comprising a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat et al., (supra)) having the sequence given as H1 in Figure 1 (SEQ ID NO:1) for CDRH1, as H2 in Figure 1 (SEQ ID NO:2) for CDRH2 or as H3 in 10 Figure 1 (SEQ ID NO:3) for CDRH3.

The antibody molecule of the first aspect of the present invention comprises at least one CDR selected from H1, H2 and H3 (SEQ ID NO:1 to SEQ ID NO:3) for the heavy chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the heavy chain variable domain.

15 In a second aspect of the present invention, there is provided an antibody molecule having specificity for human KDR, comprising a light chain wherein the variable domain comprises a CDR (as defined by Kabat et al., (supra)) having the sequence given as L1 in Figure 1 (SEQ ID NO:4) for CDRL1, as L2 in Figure 1 (SEQ ID NO:5) for CDRL2 or as L3 in Figure 1 (SEQ ID NO:6) for CDRL3.

20 The antibody molecule of the second aspect of the present invention comprises at least one CDR selected from L1, L2 and L3 (SEQ ID NO:4 to SEQ ID NO:6) for the light chain variable domain. Preferably, the antibody molecule comprises at least two and more preferably all three CDRs in the light chain variable domain.

The antibody molecules of the first and second aspects of the present invention 25 preferably have a complementary light chain or a complementary heavy chain, respectively.

Preferably, the antibody molecule of the first or second aspect of the present invention comprises a heavy chain wherein the variable domain comprises a CDR (as defined by Kabat et al., (supra)) having the sequence given as H1 in Figure 1 (SEQ ID NO:1) for CDRH1, as H2 in Figure 1 (SEQ ID NO:2) for CDRH2 or as H3 in Figure 1 (SEQ ID NO:3) for CDRH3 and a light chain wherein the variable domain comprises a CDR (as defined by Kabat et al., (supra)) having the sequence given as L1 in Figure 1

(SEQ ID NO:4) for CDRL1, as L2 in Figure 1 (SEQ ID NO:5) for CDRL2 or as L3 in Figure 1 (SEQ ID NO:6) for CDRL3.

The CDRs given in SEQ ID NOS:1 to 6 (Figure 1) referred to above are derived from a mouse monoclonal antibody VR165. The present invention also provides the mouse 5 monoclonal antibody VR165. The sequences of the variable domains of the VR165 antibody are shown in Figure 2 (SEQ ID NOS: 7 and 8). The light chain constant region of VR165 is kappa and the heavy chain constant region is IgG2a. This mouse antibody is referred to below as "the donor antibody".

In a second alternatively preferred embodiment, the antibody according to either of 10 the first and second aspects of the present invention is a chimeric mouse/human antibody molecule, referred to herein as the chimeric VR165 antibody molecule. The chimeric VR165 antibody molecule comprises the variable domains of the mouse Mab VR165 (SEQ ID NOS:7 and 8) and human constant domains. Preferably, the chimeric VR165 antibody molecule comprises the human C kappa domain (Hieter et al., *Cell*, 22, 197-207, 1980; 15 Genebank accession number J00241) in the light chain and the human gamma 4 domains (Flanagan et al., *Nature*, 300, 709-713, 1982) in the heavy chain.

In a third alternatively preferred embodiment, the antibody according to either of the first and second aspects of the present invention is a CDR-grafted antibody molecule. The term "a CDR-grafted antibody molecule" as used herein refers to an antibody 20 molecule wherein the heavy and/or light chain contains one or more CDRs from the donor antibody (e.g. a murine Mab) grafted into a heavy and/or light chain variable region framework of an acceptor antibody (e.g. a human antibody).

Preferably, such a CDR-grafted antibody has a variable domain comprising human acceptor framework regions as well as one or more of the donor CDRs referred to above.

25 When the CDRs are grafted, any appropriate acceptor variable region framework sequence may be used having regard to the class/type of the donor antibody from which the CDRs are derived, including mouse, primate and human framework regions. Examples of human frameworks which can be used in the present invention are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (Kabat et al. (supra)). For example, KOL and NEWM can 30 be used for the heavy chain, REI can be used for the light chain and EU, LAY and POM can be used for both the heavy chain and the light chain.

The preferred framework regions for the heavy chain are the human germline group 3 framework regions shown in Figure 3 (VH3-7 GL, SEQ ID NO:9). The preferred

framework regions for the light chain are the human germline sequence group 1 framework regions shown in Figure 3 (A30 GL, SEQ ID NO:10).

In a CDR-grafted antibody of the present invention, it is preferred to use as the acceptor antibody one having chains which are homologous to the chains of the donor antibody. The acceptor heavy and light chains do not necessarily need to be derived from the same antibody and may, if desired, comprise composite chains having framework regions derived from different chains.

Also, in a CDR-grafted antibody of the present invention, the framework regions need not have exactly the same sequence as those of the acceptor antibody. For instance, unusual residues may be changed to more frequently-occurring residues for that acceptor chain class or type. Alternatively, selected residues in the acceptor framework regions may be changed so that they correspond to the residue found at the same position in the donor antibody. Such changes should be kept to the minimum necessary to recover the affinity of the donor antibody. A protocol for selecting residues in the acceptor framework regions which may need to be changed is set forth in WO 91/09967.

Preferably, in a CDR-grafted antibody molecule of the present invention, if the acceptor heavy chain has human germline group 3 framework regions (shown in Figure 3) (SEQ ID NO:9), then the acceptor framework regions of the heavy chain comprise, in addition to one or more donor CDRs, donor residues at positions 77 and 93 (according to Kabat et al. (supra)).

Preferably, in a CDR-grafted antibody molecule according to the present invention, if the acceptor light chain has human group 1 framework regions (shown in Figure 3) (SEQ ID NO:10) then the acceptor framework regions of the light chain comprise donor residues at positions 36, 44, 60, 66, 69, 70 and 71 (according to Kabat et al. (supra)).

Donor residues are residues from the donor antibody, i.e. the antibody from which the CDRs were originally derived.

The antibody molecule of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as a Fab, modified Fab, di-Fab, a di-(modified Fab), Fab', F(ab')₂ or Fv fragment; a light chain or heavy chain monomer or dimer; a single chain antibody, e.g. a single chain Fv in which the heavy and light chain variable domains are joined by a peptide linker. Similarly, the heavy and light chain variable regions may be combined with other antibody domains as appropriate.

Preferably the antibody molecule of the present invention is a Fab fragment. Preferably the Fab fragment has a light chain having the sequence given as SEQ ID NO:11 (Figure 4) and a heavy chain having the sequence given as SEQ ID NO:12 (Figure 5). The amino acid sequences given in SEQ ID NO:11 and SEQ ID NO:12 are preferably encoded 5 by the nucleotide sequences given in SEQ ID NO:13 and SEQ ID NO:14, respectively (Figure 4 and Figure 5).

Alternatively, it is preferred that the antibody molecule of the present invention is a modified Fab fragment wherein the modification is the addition to the C-terminal end of its heavy chain of one or more amino acids to allow the attachment of an effector or reporter 10 molecule. Preferably, the additional amino acids form a modified hinge region containing one or two cysteine residues to which the effector or reporter molecule may be attached. Such a modified Fab fragment preferably has a light chain having the sequence given as SEQ ID NO:11 and the heavy chain having the sequence given as SEQ ID NO:12. The amino acid sequences given in SEQ ID NO:11 and SEQ ID NO:12 are preferably encoded 15 by the nucleotide sequences given in SEQ ID NO:13 and SEQ ID NO:14, respectively.

In a further alternative, it is particularly preferred that the antibody molecule of the present invention is a di-(modified Fab) fragment wherein the modification is the addition to the C-terminal end of each Fab heavy chain of one or more amino acids to allow the attachment of the chain to another such chain and to an effector or reporter molecule. 20 Preferably the additional amino acids form a modified hinge region containing one, two or three cysteine residues, for attachment to the other Fab, the effector or reporter molecules.

A preferred effector group is a polymer molecule, which may be attached to the modified Fab or di-(modified Fab) fragment to increase its half-life *in vivo*.

The polymer molecule may, in general, be a synthetic or a naturally occurring 25 polymer, for example an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide, e.g. a homo- or hetero- polysaccharide.

Particular optional substituents which may be present on the above-mentioned 30 synthetic polymers include one or more hydroxy, methyl or methoxy groups. Particular examples of synthetic polymers include optionally substituted straight or branched chain poly(ethyleneglycol), poly(propyleneglycol), poly(vinylalcohol) or derivatives thereof, especially optionally substituted poly(ethyleneglycol) such as methoxypoly(ethyleneglycol) or derivatives thereof.

Particular naturally occurring polymers include lactose, amylose, dextran, glycogen or derivatives thereof. "Derivatives" as used herein is intended to include reactive derivatives, for example thiol-selective reactive groups such as maleimides and the like. The reactive group may be linked directly or through a linker segment to the polymer. It 5 will be appreciated that the residue of such a group will in some instances form part of the product as the linking group between the antibody fragment and the polymer.

The size of the polymer may be varied as desired, but will generally be in an average molecular weight range from 500Da to 50000Da, preferably from 5000 to 40000Da and more preferably from 25000 to 40000Da. The polymer size may in 10 particular be selected on the basis of the intended use of the product.

Particularly preferred polymers include a polyalkylene polymer, such as a poly(ethyleneglycol) or, especially, a methoxypoly(ethyleneglycol) or a derivative thereof, and especially with a molecular weight in the range from about 25000Da to about 40000Da.

15 Each polymer molecule attached to the modified antibody fragment may be covalently linked to the sulphur atom of a cysteine residue located in the fragment. The covalent linkage will generally be a disulphide bond or, in particular, a sulphur-carbon bond.

Where desired, the antibody fragment may have one or more other effector or 20 reporter molecules attached to it. The effector or reporter molecules may be attached to the antibody fragment through any available amino acid side-chain or terminal amino acid functional group located in the fragment, for example any free amino, imino, hydroxyl or carboxyl group.

An activated polymer may be used as the starting material in the preparation of 25 polymer-modified antibody fragments as described above. The activated polymer may be any polymer containing a thiol reactive group such as an α -halocarboxylic acid or ester, e.g. iodoacetamide, an imide, e.g. maleimide, a vinyl sulphone or a disulphide. Such starting materials may be obtained commercially (for example from Shearwater Polymers Inc., Huntsville, AL, USA) or may be prepared from commercially available starting 30 materials using conventional chemical procedures.

As regards attaching poly(ethyleneglycol) (PEG) moieties, reference is made to "Poly(ethyleneglycol) Chemistry, Biotechnical and Biomedical Applications", 1992, J. Milton Harris (ed), Plenum Press, New York, "Poly(ethyleneglycol) Chemistry and

Biological Applications", 1997, J. Milton Harris and S. Zalipsky (eds), American Chemical Society, Washington DC and "Bioconjugation Protein Coupling Techniques for the Biomedical Sciences", 1998, M. Aslam and A. Dent, Grove Publishers, New York.

Where it is desired to obtain an antibody fragment linked to an effector or reporter molecule, this may be prepared by standard chemical or recombinant DNA procedures in which the antibody fragment is linked either directly or via a coupling agent to the effector or reporter molecule either before or after reaction with the activated polymer as appropriate. Particular chemical procedures include, for example, those described in WO 93/06231, WO 92/22583, WO 90/09195 and WO 89/01476. Alternatively, where the effector or reporter molecule is a protein or polypeptide the linkage may be achieved using recombinant DNA procedures, for example as described in WO 86/01533 and EP-A-0392745.

Preferably, the modified Fab fragment or di-Fab of the present invention is PEGylated (i.e. has PEG (poly(ethyleneglycol)) or mPEG (methoxypoly(ethyleneglycol)) covalently attached thereto) according to the methods disclosed in EP-A-0948544 and EP-A-1090037. Preferably the antibody molecule of the present invention is a PEGylated modified Fab fragment as shown in Figure 6 or a PEGylated di-(modified Fab) fragment. As shown in Figure 6, the modified Fab fragment has a maleimide group covalently linked to a single thiol group in a modified hinge region. A lysine residue is covalently linked to the maleimide group. To each of the amine groups on the lysine residue is attached a methoxypoly(ethyleneglycol) polymer having a molecular weight of approximately 20,000 Da. The total molecular weight of the entire effector molecule is therefore approximately 40,000 Da. Similarly each mPEG may be linked to a lysine residue covalently attached to a bis-maleimide linker as described in EP-A-1090037 to form a PEGylated di-(modified Fab) according to the invention.

Preferably, in the compound shown in Figure 6, the heavy chain of the antibody part has the sequence given as SEQ ID NO:12 and the light chain has the sequence given in SEQ ID NO:11.

The constant region domains of the antibody molecule of the present invention, if present, may be selected having regard to the proposed function of the antibody molecule, and in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgD, IgE, IgG or IgM domains. In particular, human IgG constant region domains may be used, especially of the IgG1 and IgG3 isotypes when

the antibody molecule is intended for therapeutic uses and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simply blocking KDR ligation by VEGF.

5 Also, the antibody molecule of the present invention may have an effector or a reporter molecule attached to it. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, procedures of recombinant DNA technology may be used to produce an antibody molecule in which the Fc fragment (CH2, CH3 and hinge domains), the CH2 and
10 CH3 domains or the CH3 domain of a complete immunoglobulin molecule has (have) been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The antibody molecule of the present invention preferably has a binding affinity of 0.4×10^{-10} M. Preferably, the antibody molecule of the present invention comprises the
15 heavy chain variable domain gH3 (SEQ ID NO:15) and the light chain variable domain gL3 (SEQ ID NO:16). The sequences of the variable domains of these light and heavy chains are shown in Figure 7.

The present invention also relates to variants of the antibody molecule of the present invention, which have an improved affinity for KDR. Such variants can be
20 obtained by a number of affinity maturation protocols including mutating the CDRs (Yang et al., J. Mol. Biol., 254, 392-403, 1995), chain shuffling (Marks et al., Bio/Technology, 10, 779-783, 1992), use of mutator strains of E. coli (Low et al., J. Mol. Biol., 250, 359-368, 1996), DNA shuffling (Patten et al., Curr. Opin. Biotechnol., 8, 724-733, 1997), phage display (Thompson et al., J. Mol. Biol., 256, 77-88, 1996) and sexual PCR (Crameri et al., Nature, 391, 288-291, 1998). Vaughan et al. (supra) discusses these methods of
25 affinity maturation.

The present invention also provides a DNA sequence encoding the heavy and/or light chain(s) of the antibody molecule of the present invention, for example as described in the figures herein.

30 The DNA sequence of the present invention may comprise synthetic DNA, for instance produced by chemical processing, cDNA, genomic DNA or any combination thereof.

The present invention also relates to a cloning or expression vector comprising one or more DNA sequences of the present invention. Preferably, the cloning or expression vector comprises two DNA sequences, encoding the light chain and the heavy chain of the antibody molecule of the present invention, respectively.

5 In a preferred embodiment, the present invention provides an *E. coli* expression vector comprising a DNA sequence of the present invention. Preferably the expression vector is pTTOD(CDP791) as shown schematically in Figure 8.

10 General methods by which the vectors may be constructed, transfection methods and culture methods are well known to those skilled in the art. In this respect, reference is made to "Current Protocols in Molecular Biology", 1999, F. M. Ausubel (ed), Wiley Interscience, New York and the Maniatis Manual produced by Cold Spring Harbor Publishing.

15 DNA sequences which encode the antibody molecule of the present invention can be obtained by methods well known to those skilled in the art. For example, DNA sequences coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequences or on the basis of the corresponding amino acid sequences.

20 DNA coding for acceptor framework sequences is widely available to those skilled in the art and can be readily synthesised on the basis of their known amino acid sequences.

25 Standard techniques of molecular biology may be used to prepare DNA sequences coding for the antibody molecule of the present invention. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate.

30 Any suitable host cell/vector system may be used for expression of the DNA sequences encoding the antibody molecule of the present invention. Bacterial, for example *E. coli*, and other microbial systems may be used, in part, for expression of antibody fragments such as Fab, di-(modified Fab) and F(ab')₂ fragments, and especially Fv fragments and single chain antibody fragments, for example, single chain Fvs. Eukaryotic, e.g. mammalian, host cell expression systems may be used for production of larger antibody molecules, including complete antibody molecules. Suitable mammalian host cells include CHO, myeloma or hybridoma cells.

The present invention also provides a process for the production of an antibody molecule according to the present invention comprising culturing a host cell comprising a vector of the present invention under conditions suitable for leading to expression of protein from DNA encoding the antibody molecule of the present invention, and isolating the antibody molecule.

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Preferably the process for the production of the antibody molecule of the present invention comprises culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of the present invention under conditions suitable for leading to expression of protein from the DNA sequence and isolating the antibody molecule. The antibody molecule may be secreted from the cell or targeted to the periplasm by suitable signal sequences. Alternatively, the antibody molecules may accumulate within the cell's cytoplasm. Preferably the antibody molecule is targeted to the periplasm. Depending on the antibody molecule being produced and the process used, it is desirable to allow the antibody molecules to refold and adopt a functional conformation. Procedures for allowing antibody molecules to refold are well known to those skilled in the art.

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The antibody molecule may comprise only a heavy or light chain polypeptide, in which case only a heavy chain or light chain polypeptide coding sequence needs to be used to transfet the host cells. For production of products comprising both heavy and light chains, the cell line may be transfected with two vectors, a first vector encoding a light chain polypeptide and a second vector encoding a heavy chain polypeptide. Alternatively, a single vector may be used, the vector including sequences encoding light chain and heavy chain polypeptides.

The present invention also provides a therapeutic or diagnostic composition comprising an antibody molecule of the present invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The present invention also provides a process for preparation of a therapeutic or diagnostic composition comprising admixing the antibody molecule of the present invention together with a pharmaceutically acceptable excipient, diluent or carrier.

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The antibody molecule may be the sole active ingredient in the therapeutic or diagnostic composition or may be accompanied by other active ingredients including other antibody ingredients, for example anti-T cell, anti-IFNy or anti-LPS antibodies, or non-antibody ingredients such as xanthines.

The pharmaceutical compositions should preferably comprise a therapeutically effective amount of the antibody of the invention. The term "therapeutically effective amount" as used herein refers to an amount of a therapeutic agent needed to treat, ameliorate or prevent a targeted disease or condition, or to exhibit a detectable therapeutic 5 or preventative effect. For any antibody, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually in rodents, rabbits, dogs, pigs or primates. The animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

10 The precise effective amount for a human subject will depend upon the severity of the disease state, the general health of the subject, the age, weight and gender of the subject, diet, time and frequency of administration, drug combination(s), reaction sensitivities and tolerance/response to therapy. This amount can be determined by routine experimentation and is within the judgement of the clinician. Generally, an effective dose 15 will be from 0.01 mg/kg to 50 mg/kg, preferably 0.1 mg/kg to 20 mg/kg, more preferably about 15 mg/kg.

Compositions may be administered individually to a patient or may be administered in combination with other agents, drugs or hormones.

The dose at which the antibody molecule of the present invention is administered 20 depends on the nature of the condition to be treated, the degree to which the level of VEGF to be neutralised is, or is expected to be, raised above a desirable level, and on whether the antibody molecule is being used prophylactically or to treat an existing condition. The dose will also be selected according to the age and condition of the patient.

Thus, for example, where the product is for treatment or prophylaxis of a chronic 25 inflammatory disease, such as rheumatoid arthritis, suitable doses of the antibody molecule of the present invention lie in the range of between 0.5 and 50 mg/kg, more preferably between 1 and 20 mg/kg and most preferably about 15 mg/kg. The frequency of dose will depend on the half-life of the antibody molecule and the duration of its effect.

If the antibody molecule has a short half-life (e.g. 2 to 10 hours) it may be 30 necessary to give one or more doses per day. Alternatively, if the antibody molecule has a long half life (e.g. 2 to 15 days) it may only be necessary to give a dosage once per day, per week or even once every 1 or 2 months.

A pharmaceutical composition may also contain a pharmaceutically acceptable carrier for administration of the antibody. The carrier should not itself induce the production of antibodies harmful to the individual receiving the composition and should not be toxic. Suitable carriers may be large, slowly metabolised macromolecules such as 5 proteins, polypeptides, liposomes, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers and inactive virus particles.

Pharmaceutically acceptable salts can be used, for example mineral acid salts, such as hydrochlorides, hydrobromides, phosphates and sulphates, or salts of organic acids, such as acetates, propionates, malonates and benzoates.

10 Pharmaceutically acceptable carriers in therapeutic compositions may additionally contain liquids such as water, saline, glycerol and ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents or pH buffering substances, may be present in such compositions. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries and 15 suspensions, for ingestion by the patient.

Preferred forms for administration include forms suitable for parenteral administration, e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain 20 formulatory agents, such as suspending, preservative, stabilising and/or dispersing agents. Alternatively, the antibody molecule may be in dry form, for reconstitution before use with an appropriate sterile liquid.

Once formulated, the compositions of the invention can be administered directly to the subject. The subjects to be treated can be animals. However, it is preferred that the 25 compositions are adapted for administration to human subjects.

The pharmaceutical compositions of this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, transcutaneous (for example, see WO98/20734), subcutaneous, intraperitoneal, intranasal, enteral, topical, 30 sublingual, intravaginal or rectal routes. Hyposprays may also be used to administer the pharmaceutical compositions of the invention. Typically, the therapeutic compositions may be prepared as injectables, either as liquid solutions or suspensions. Solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection may also be prepared.

Direct delivery of the compositions will generally be accomplished by injection, subcutaneously, intraperitoneally, intravenously or intramuscularly, or delivered to the interstitial space of a tissue. The compositions can also be administered into a lesion. Dosage treatment may be a single dose schedule or a multiple dose schedule.

5 It will be appreciated that the active ingredient in the composition will be an antibody molecule. As such, it will be susceptible to degradation in the gastrointestinal tract. Thus, if the composition is to be administered by a route using the gastrointestinal tract, the composition will need to contain agents which protect the antibody from degradation but which release the antibody once it has been absorbed from the
10 gastrointestinal tract.

A thorough discussion of pharmaceutically acceptable carriers is available in Remington's Pharmaceutical Sciences (Mack Publishing Company, N.J. 1991).

It is also envisaged that the antibody of the present invention will be administered by use of gene therapy. In order to achieve this, DNA sequences encoding the heavy and
15 light chains of the antibody molecule under the control of appropriate DNA components are introduced into a patient such that the antibody chains are expressed from the DNA sequences and assembled *in situ*.

The present invention also provides the antibody molecule of the present invention for use in treating a disease in which VEGF and/or KDR are implicated.

20 The present invention further provides the use of the antibody molecule according to the present invention in the manufacture of a medicament for the treatment in which VEGF and/or KDR are implicated.

The antibody molecule of the present invention may be utilised in any therapy where it is desired to reduce the level of biologically active KDR present in the human or
25 animal body. The VEGF may be circulating in the body or present in an undesirably high level localised at a particular site in the body.

For example, VEGF (and therefore KDR) has been implicated in a number of pathological conditions including inflammation, psoriasis, rheumatoid arthritis and tumour growth and metastasis.

30 The present invention also provides a method of treating human or animal subjects suffering from or at risk of a disorder in which VEGF and/or KDR are implicated, the method comprising administering to the subject an effective amount of the antibody molecule of the present invention.

The antibody molecule of the present invention may also be used in diagnosis, for example in the *in vivo* diagnosis and imaging of disease states involving elevated levels of KDR.

The present invention is further described by way of illustration only in the 5 following examples which refer to the accompanying Figures, in which:

Figure 1 shows the CDR sequences of the heavy and light chain V-regions of the VR165 mouse monoclonal antibody gene (SEQ ID NOS 1-6).

Figure 2 shows the protein sequence of mouse monoclonal antibody VR165 VH 10 and VL domains (SEQ ID NO:7 and SEQ ID NO:8).

Figure 3 shows the V-region protein sequences chosen as human germline acceptor frameworks. VH3-7 GL is a human germline VH gene (SEQ ID NO:9). A30 GL refers to the human VL germline sequence A30 gene (SEQ ID NO:10). In each case the germline sequence of framework 4 is provided by the human germline J_H4 and J_K1 respectively.

15 Figure 4 shows the amino acid and nucleotide sequence of the CDP791 Fab light chain (SEQ ID NO:11 and SEQ ID NO:13).

Figure 5 shows the amino acid and nucleotide sequence of the CDP791 Fab heavy chain (SEQ ID NO:12 and SEQ ID NO:14).

Figure 6 shows the structure of a modified Fab fragment derived from antibody 20 VR165 covalently linked via a cysteine residue to a lysyl-maleimide linker wherein each amino group on the lysyl residue has covalently attached to it a methoxy PEG residue wherein n is about 420;

Figure 7 shows the protein sequences for the optimised CDR-grafted VH and VL domains gene (SEQ ID NO:15 and SEQ ID NO:16).

25 Figure 8 shows the optimised pTTOD(CDP791) plasmid which contains the IGS-2 variant between grafts gL3 and gH3.

Figure 9 shows the protein sequence of the designed VH and VL grafts (gH1-3 and gL1-3, SEQ ID NOS 17-22). Graft gH1 contains no murine framework residues. Graft gH2 contains murine residues at positions 77 and 93 (Kabat numbering). Both T and S are 30 common in human germline sequences at position 77, so the inclusion of T is still consistent with a human residue. The V at position 93 is likely to be important at the VH/VL interface. The inclusion of the human residues at 60 and 62 represents the changes to the C-terminal portion of CDR-H2. Graft gL2 contains murine residues at positions 60,

66, 69, 70 and 71 (Kabat numbering). Graft gL3 contains additional murine residues at positions 36 and 44).

Figure 10 shows the design of genes encoding the gH1 and gL1 grafts (SEQ ID NO:23 and SEQ ID NO:24).

5 Figure 11 shows the oligonucleotides used to assemble the genes encoding for the gL1 and gH1 grafts (SEQ ID NOS:25-40).

Figure 12 shows plasmids pCR2.1(gH1) and pCR2.1(gL1) which contain the gH1 and gL1 grafts, respectively.

10 Figure 13 shows oligonucleotide cassettes used in the construction of grafts gH2, gH3, gL2 and gL3 (SEQ ID NOS:41-44).

Figure 14 shows oligonucleotide pairs used in the construction of grafts gH2, gH3, gL2 and gL3 (SEQ ID NOS:45-52).

Figure 15 shows plasmids pGamma4 and pMR10.1 into which the VH and VL grafts, respectively, were sub-cloned to enable expression in CHO cell lines.

15 Figure 16 shows E.coli Fab' expression plasmid pTTOD, in this case containing the IGS-3 sequence.

Figure 17 shows the nucleotide sequence of the three IGS regions tested (SEQ ID NOS:53-55).

20 Figure 18 shows the results of the Fab' fermentation comparison of IGS performance.

Figure 19 shows the coding and flanking sequence of the CDP791 Fab' fragment (SEQ ID NO:56).

Figure 20 shows the radioimmunoassay results, in which the antibody fragments are tested for blocking of VEGF binding to KDR.

25 Figure 21 shows the amino acid sequence of the entire heavy chain of the gH3-grafted VR165-derived monoclonal antibody (SEQ ID NO:57).

EXAMPLES**Monoclonal Antibody production and selection**

An in-house immunisation program was initiated to select an antibody to human KDR that potently blocks the interaction with its ligand VEGF. Mice were immunised with a variety of immunogens including CHO cells transfected with full length human KDR, purified human KDR-human Fc fusion proteins and DNA encoding these fusion proteins. From a total of 19 fusions from animals immunised with cellular/protein immunogens, and 4 fusions from animals immunised with DNA, approximately 23,000 wells were screened in a primary ELISA format for binding to human 7-domain KDR-Fc. About 800 antibodies were then subjected to a secondary screen, a radioimmunoassay measuring blocking of 125-I VEGF binding to human 7-domain KDR-Fc. A tertiary screen measured the blocking of VEGF stimulated Tissue Factor release from human umbilical vein endothelial cells (HUVECs). From this screening cascade, antibody VR165 was selected (data not shown).

Gene Cloning of VR165

RNA was prepared from hybridoma cells expressing VR165 and was reverse transcribed to DNA. This was then used as the template for a series of PCR reactions to amplify the V-region sequences. Degenerate primer pools designed to anneal within the conserved heavy and light chain signal sequences were used as forward primers, while primers encoding the framework 4 / C-region junction served as reverse primers. In this way, the V-region genes of both the heavy and light chain were amplified and then cloned and sequenced. The DNA sequences were translated to give the VR165 V-region amino acid sequence which was verified by reference to the protein sequence determined by N-terminal sequencing. The murine V-region genes were then sub-cloned into the expression vectors pMR10.1 and pGamma-4. These are separate vectors for expression of the light and heavy chain respectively containing genomic DNA encoding constant region genes for human kappa light chain and gamma-4 heavy chain. Co-transfection into CHO cells generates chimeric VR165 antibody.

Design of CDR-grafted sequences

VR165 was CDR-grafted onto human frameworks in order to reduce potential immunogenicity and to facilitate *E. coli* expression. Human germline acceptor frameworks were chosen from sub-group VHIII and VL1. The heavy chain acceptor framework is the 5 human germline sequence VH3-7, with framework 4 coming from this portion of the human JH-region germline JH4. The light chain acceptor framework is the human germline sequence A30, with framework 4 coming from this portion of the human JK-region germline JK1. The alignment shows that there are 15 framework differences between the donor and acceptor heavy chains. At each of these positions an analysis was 10 made of the potential of that residue to contribute to antigen binding; if considered important, the murine donor residue was retained. The light chain alignment shows that there are 24 framework differences between the donor and acceptor sequences. The potential of the murine residue to contribute to antigen binding was again analysed. In this way, three VH grafts were designed and three VL grafts (Figure 9, SEQ ID NOS:17-22). 15 In each case graft 1 represents a graft without murine framework residues. Grafts 2 and 3 contain murine framework residues at the positions shown. Graft gH3 also contains additional human residues at the C-terminal end of CDR-H2. This portion of the CDR is not at the antigen binding surface. Genes were designed to encode the grafted sequences, using codons frequently used in *E. coli* genes and avoiding 'rare' *E. coli* codons (Wada et 20 al., Nucl. Acids Res., 19, 1981-86, 1991). Restriction sites were introduced into the DNA sequence at intervals to facilitate further genetic manipulation. Figure 10 shows the design of genes for gH1 and gL1 (SEQ ID NO:23 and SEQ ID NO:24). The oligonucleotides used to construct the genes are shown in figure 11 (SEQ ID NOS:25-40).

25 Construction of genes for grafted sequences

A PCR assembly technique was employed to construct the CDR-grafted gH1 and gL1 V-region genes. Reaction volumes of 100 μ l were set up containing 10mM Tris-HCl pH 8.3, 1.5mM MgCl₂, 50mM KCl, 0.001% gelatin, 0.25 mM each deoxyribonucleoside triphosphate, 1 pmole each of the 'internal' primers (F2, F3, F4, R2, R3, R4), 10 pmole 30 each of the 'external' primers (F1, R1), and 1 unit of Taq polymerase (AmpliTaq, Applied BioSystems, catalogue no. N808-0171). PCR cycle parameters were 94°C for 1 minute, 55°C for 1 minute and 72°C for 1 minute, for 30 cycles. The reaction products were then run on a 1.5% agarose gel, excised and recovered using QIAGEN spin columns (QIAquick

gel extraction kit, cat no. 28706). The DNA was eluted in a volume of 30 μ l. Aliquots (1 μ l) of the gH1 and gL1 DNA were then cloned into the InVitrogen TOPO TA cloning vector pCR2.1 TOPO (catalogue no. K4500-01) according to the manufacturer's instructions. This non-expression vector served as a cloning intermediate to facilitate 5 sequencing of a large number of clones. DNA sequencing using vector specific primers was used to identify correct clones containing gH1 and gL1, creating plasmids pCR2.1(gH1), and pCR2.1(gL1) (see Figure 12).

An oligonucleotide cassette replacement method was used to create the humanised 10 grafts gH2 and gL2. Figure 13 shows the design of the oligonucleotide cassettes (SEQ ID NO:41 and SEQ ID NO:43). To construct each variant, the vector (pCR2.1(gH1) or pCR2.1(gL1)) was cut with the restriction enzymes shown (Figure 13, restriction sites underlined), the large vector fragment was gel purified from agarose and was used in ligation with the oligonucleotide cassette. Figure 14 shows the sequences of the 15 oligonucleotides used in the cassettes (SEQ ID NOS:45-46 and SEQ ID NOS:49-50). Pairs were annealed together mixing at a concentration of 0.5 pmoles/ μ l in a volume of 200 μ l containing 12.5mM TrisHCl pH 7.5, 2.5 mM MgCl₂, 25 mM NaCl, 0.25mM dithioerythritol, and heating to 95°C for 3 minutes in a waterbath (volume 500 ml) then allowed to slow-cool to room temperature. The annealed oligonucleotide cassette was then 20 diluted ten-fold in water before ligation into the appropriately cut vector. DNA sequencing was used to confirm the correct sequence, creating plasmids pCR2.1(gH2) and pCR2.1(gL2).

Variants gH3 and gL3 were constructed in similar fashion from gH2 and gL2. The 25 cassettes and oligonucleotides are shown in Figures 13 and 14 (SEQ ID NO:42 and SEQ ID NO:44, SEQ ID NOS:47-48 and SEQ ID NOS:51-52). Construction of gL3 required a modified strategy because of the existence of Pvul sites in the pCR2.1 vector backbone. Cleavage of pCR2.1(gL2) with AatII and SfuI created a vector molecule into which was 30 ligated the Pvul-AatII annealed cassette plus a 225 base pair SfuI-Pvul fragment also prepared from pCR2.1(gL2). DNA sequencing was used to confirm the correct sequence, creating plasmids pCR2.1(gH3) and pCR2.1(gL3).

Each of the 3 heavy chain grafts was then sub-cloned into the expression vector pGamma-4 as HindIII-ApaI fragments. Each of the 3 light chain grafts was sub-cloned into the light chain expression vector pMR10.1 as SfuI-BsiWI fragments. Figure 15 shows maps of these expression vectors. Antibodies were expressed transiently by co-
5 transfection into CHO cells. All combinations of grafted chain and chimeric chain were expressed and compared against the double chimeric antibody.

Binding was assessed in a KDR binding ELISA, in a radioimmunoassay of inhibition of labeled VEGF binding to KDR and in a BIAcore assay of KDR binding. All
10 the grafted forms performed well in the ELISA and radioimmunoassay, showing activity similar to the chimeric. From the BIAcore analysis, graft gL3gH3 was selected as the optimum (data not shown), and is henceforth referred to as g165.

Construction of plasmid pTTOD

15 Plasmid pTTO-1 was constructed as follows.

(a) Replacement of the pTTQ9 Polylinker

Plasmid pTTQ9 was obtained from Amersham. An aliquot (2 µg) was digested with restriction enzymes SalI and EcoRI, the digest was run on a 1% agarose gel and the
20 large DNA fragment (4520 bp) was purified. Two oligonucleotides were synthesized which, when annealed together, encode the OmpA polylinker region. This sequence has cohesive ends which are compatible with the SalI and EcoRI ends generated by restriction of pTTQ9. By cloning this oligonucleotide 'cassette' into the pTTQ9 vector, the SalI site is not regenerated, but the EcoRI site is maintained. The cassette encodes the first 13 amino
25 acids of the signal sequence of the *E. coli* outer-membrane protein Omp-A, preceded by the Shine Dalgarno ribosome binding site of the OmpA gene. In addition restriction sites for enzymes XbaI, MunI, StyI and SphI are present. The MunI and StyI sites are within the coding region of the OmpA signal sequence and are intended as the 5' cloning sites for insertion of genes. The two oligonucleotides which make up this cassette were annealed
30 together by mixing at a concentration of 5 pmoles/µl and heating in a waterbath to 95°C for 3 minutes, then slow cooling to room temperature. The annealed sequence was then ligated into the SalI / EcoRI cut pTTQ9. The resulting plasmid intermediate, termed pTQOmp, was verified by DNA sequencing.

(b) Fragment Preparation and Ligation

Plasmid pTTO-1 was constructed by ligating one DNA fragment from plasmid pACYC184 to two fragments generated from pTQOmp. Plasmid pACYC184 was 5 obtained from New England Biolabs. An aliquot (2 µg) was digested to completion with restriction enzyme StyI, then treated with Mung Bean Nuclease; this treatment creates blunt ends by cutting back 5' base overhangs. Following phenol extraction and ethanol precipitation, the DNA was restricted with enzyme Pvull, generating fragments of 2348, 1081, 412 and 403 bp. The 2348 bp fragment was purified after agarose gel 10 electrophoresis. This fragment encodes the tetracycline resistance marker and the p15A origin of replication. The fragment was then treated with calf intestinal alkaline phosphatase to remove 5' terminal phosphates, thereby preventing the self-ligation of this molecule.

An aliquot (2 µg) of plasmid pTQOmp was digested with enzymes SspI and EcoRI, 15 and the 2350 bp fragment was purified from unwanted fragments of 2040 bp and 170 bp following agarose gel electrophoresis; this fragment encodes the transcriptional terminator region and the lacI^q gene. Another aliquot (2 µg) of pTQOmp was digested with EcoRI and XmnI, generating fragments of 2289, 1670, 350 and 250 bp. The 350 bp fragment, encoding the tac promoter, OmpA signal sequence and multicloning site, was gel purified.

20 The three fragments were then ligated, using approximately equimolar amounts of each fragment, to generate the plasmid pTTO-1. All cloning junctions were verified by DNA sequencing.

(c) Production of Plasmid pTTOD

25 Plasmid pTTOD was derived from pTTO-1 by removal of backbone restriction sites for Pvull (3 sites), EcoRV (2 sites) and Apal (1 site). These changes were made to simplify Fab' coding strategies. In making these changes the coding protein sequence of the lacI^q gene and tetracycline resistance gene were not altered, although 'silent' changes were made at the DNA level. A PCR strategy was used, in which primers bearing 'silent' 30 changes which removed these restriction sites were designed and used to amplify sections of the parent plasmid (pTTO-1). Flanking restriction sites (unaltered) were then used to replace sequences in the parent plasmid with these modified sequences. By this multi-stage process plasmid pTTOD was created. Transfer of existing Fab' genes within vector

pTTO into pTTOD was achieved using the unique PstI and EcoRI sites which flank the genes, creating pTTOD(Fab').

Insertion of g165 V-region genes into *E. coli* Fab' expression plasmid pTTOD

5 The starting point for insertion of g165 sequences was 3 vectors for expression of an irrelevant Fab', pTTOD(Fab' IGS-1), pTTOD(Fab' IGS-2) and pTTOD(Fab' IGS-3) (for example, see Figure 16). These differ only in the so-called IGS or intergenic sequence which separates the light chain gene from the heavy chain gene. These IGS regions are shown in Figure 17 (SEQ ID NOS:53-55). Cloning of the g165 sequences into these
10 vectors was performed as a 2-stage process. First the light chain was restricted from pCR2.1(gL3) as a EcoRV-BsiWI fragment (395 bp) and inserted into the large vector fragment from EcoRV-BsiWI digestion of pTTOD(Fab' IGS-1), pTTOD(Fab' IGS-2) and pTTOD(Fab' IGS-3). This created the cloning intermediates pTTOD(g165L IGS-1), pTTOD(g165L IGS-2) and pTTOD(g165L IGS-3). These cloning intermediates were then
15 cut with PvuII and ApaI, the large vector fragment was purified and the 435 bp PvuII-ApaI fragment from pCR2.1(gH3) was inserted. This created the 3 Fab' expression plasmids pTTOD(g165 IGS-1), pTTOD(g165 IGS-2) and pTTOD(g165 IGS-3).

20 These plasmids were transformed into the host strain W3110 and expression of Fab' by these 3 plasmids was compared in shake flasks and in the fermenter. Figure 18 shows the results of a fermenter comparison, clearly demonstrating the superior performance of the IGS-2 variant.

25 Plasmid pTTOD(g165 IGS-2) was retermed pTTOD(CDP791). The plasmid map of this construct is shown in Figure 8. Figure 19 shows the full DNA and protein sequence of the coding region of the Fab' in this vector, plus some of the 5' and 3' flanking sequence (SEQ ID NO:56).

PEGylation of CDR-Grafted, VR165-based Modified Fab

30 The purified modified Fab is site-specifically conjugated with a branched molecule of mPEG. This is achieved by activation of a single cysteine residue in a truncated hinge region of the modified Fab, followed by reaction with (mPEG)-lysyl maleimide as previously described (A.P. Chapman et al., *Nature Biotechnology* 17, 780-783, 1999). The

PEGylated molecule is shown in Figure 6. Alternatively, reaction of the activated Fab with (mPEG)-lysyl bis-maleimide as described in EP-A-1090037 yields a PEGylated di-(modified Fab), hereinafter referred to as DFM.

5 **BIAcore activities of naked and PEGylated fragments**

7 Ig-domain human KDR fused to human Fc was captured on a chip coated with anti-Fc, and the various fragments of the CDR grafted antibody g165 and the murine parent antibody VR165 were passed over permitting affinity determination. The table below summarises the results obtained. In this assay format, there is an advantage of divalency as shown by the lower off rates (K_d) of the divalent species. The affinity of the grafted DFM is very similar to the murine IgG, with the DFM-PEG showing a minor reduction of affinity. The KD of the g165 DFM-PEG molecule is approximately $4 \times 10^{-11} M$ in this assay.

15 **Table 1: BIAcore activities of naked and PEGylated fragments**

a-KDR	$K_a e^5$	$K_d e^{-4}$	$K_D e^{-10}$
DFM	21.6	0.64	0.29
DFM-PEG40	15.5	0.64	0.41
mIgG	19.8	0.60	0.30
FAB	13.6	12.4	9.1
FAB-PEG40	11.0	11.8	10.7

Radioimmunoassay

The ability of the fragments to block VEGF binding to KDR was measured in a radioimmunoassay. Polyclonal anti Fc was used to capture 7 Ig-domain KDR fused to 5 human Fc in a microtitre plate, antibody or fragment was added followed by 125-I labeled VEGF-165. Results of this assay are shown in Figure 20. Again in this assay set-up there is an advantage of divalency, demonstrated by the superior blocking performance of the DFM over the Fab'. The DFM-PEG construct shows a minor reduction of activity compared to the naked DFM, as was seen in the BIACore study.

10

Cell based assays

The molecule g164 DFM PEG also demonstrated activity in cell based assays. Its ability to block VEGF stimulation of KDR was demonstrated via inhibition of tissue factor release by human umbilical vein endothelial cells (see Clauss et al., *J. Biol. Chem.*, 271, 17629-15 17634, 1996). Activity was also demonstrated via inhibition of VEGF mediated Ca^{2+} mobilisation in human microvascular endothelial cells (see Cunningham et al., *Am. J. Physiol.*, 276, C176-181, 1999).

It should be understood that the above-described examples are merely exemplary 20 and do not limit the scope of the present invention as defined in the following claims.

CLAIMS

1. An antibody molecule having specificity for human KDR, comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given as H1 in Figure 1 (SEQ ID NO:1) for CDRH1, as H2 in Figure 1 (SEQ ID NO:2) or as H3 in Figure 1 (SEQ ID NO:3) for CDRH3.
2. An antibody molecule having specificity for human KDR, comprising a light chain wherein the variable domain comprises a CDR having the sequence given as L1 in Figure 1 (SEQ ID NO:4) for CDRL1, as L2 in Figure 1 (SEQ ID NO:5) for CDRL2 or as L3 in Figure 1 (SEQ ID NO:6) for CDRL3.
3. The antibody molecule of claim 1 or claim 2 comprising a heavy chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:1 for CDRH1, SEQ ID NO:2 for CDRH2 or SEQ ID NO:3 for CDRH3 and a light chain wherein the variable domain comprises a CDR having the sequence given in SEQ ID NO:4 for CDRL1, SEQ ID NO:5 for CDRL2 or SEQ ID NO:6 for CDRL3.
4. The antibody molecule of claim 3, which comprises SEQ ID NO:1 for CDRH1, SEQ ID NO: 2 for CDRH2, SEQ ID NO:3 for CDRH3, SEQ ID NO:4 for CDRL1, SEQ ID NO:5 for CDRL2 and SEQ ID NO:6 for CDRL3.
5. The antibody molecule of any one of claims 1 to 4, which is a CDR-grafted antibody molecule.

25

6. The antibody molecule of claim 5, wherein the variable domain comprises human acceptor framework regions and non-human donor CDRs.
7. The antibody molecule of claim 6, wherein the human acceptor framework regions of the variable domain of the heavy chain are based on a human germline group 3 framework sequence and comprise non-human donor residues at positions 77 and 93.

8. The antibody molecule of claim 6 or claim 7, wherein the human acceptor framework regions of the variable domain of the light chain are based on human germline group 1 framework sequence and comprise non-human donor residues at positions 36, 44, 60, 66, 69, 70 and 71.

5

9. The antibody molecule of any one of claims 1 to 8, comprising the heavy chain variable region gH3 (SEQ ID NO:15) and light chain variable region gL3 (SEQ ID NO:16).

10 10. The antibody molecule of any one of claims 1 to 9 which is a Fab fragment.

11. The antibody molecule of claim 10, which is a Fab fragment comprising a light chain having the sequence given in SEQ ID NO:11 and a heavy chain having the sequence given in SEQ ID NO:12.

15

12. The antibody molecule of any one of claims 1 to 9, which is a modified Fab fragment having at the C-terminal end of its heavy chain one or more amino acids to allow attachment of an effector or reporter molecule.

20 13. The antibody molecule of claim 12, wherein the additional amino acids form a modified hinge region containing one or two cysteine residues to which the effector or reporter molecule may be attached.

25 14. The antibody molecule of claim 12, which is a modified Fab or di-Fab fragment comprising a light chain having the sequence given in SEQ ID NO:11 and a heavy chain having the sequence given in SEQ ID NO:12.

15. An antibody molecule having specificity for human KDR, having a light chain comprising the sequence given in SEQ ID NO:11.

30

16. An antibody molecule having specificity for human KDR, having a heavy chain comprising the sequence given in SEQ ID NO:57

17. An antibody molecule having specificity for human KDR, having a light chain comprising the sequence given in SEQ ID NO:12 and a heavy chain comprising the sequence given in SEQ ID NO:57

5 18. A variant of the antibody molecule of any one of claims 1 to 17, which has an improved affinity for KDR.

19. The variant of claim 18 which is obtained by an affinity maturation protocol.

10 20. The antibody of any one of claims 1 to 4 which is murine anti-KDR monoclonal antibody VR165.

21. The antibody molecule of any one of claims 1 to 4, which is a chimeric antibody molecule comprising the light and heavy chain variable domains of the monoclonal 15 antibody of claim 26.

22. A compound comprising the antibody molecule of any one of claims 10 to 14 having covalently attached to an amino acid at or towards the C-terminal end of its heavy chain an effector or reporter molecule.

20 23. The compound of claim 22, which comprises an effector molecule.

24. The compound of claim 23, wherein the effector molecule comprises one or more polymers.

25 25. The compound of claim 24, wherein the one or more polymers is/are an optionally substituted straight or branched chain polyalkylene, polyalkenylene or polyoxyalkylene polymer or a branched or unbranched polysaccharide.

30 26. The compound of claim 25, wherein the one or more polymers is/are a methoxypoly(ethyleneglycol).

27. A compound comprising the antibody molecule of claim 12 having attached to one of the cysteine residues at the C-terminal end of the heavy chain a lysyl-maleimide or lysyl bis-maleimide group wherein each amino group of the lysyl residue has covalently linked to it a methoxypoly(ethyleneglycol) residue having a molecular weight of about 20,000 Da.

5

28. A DNA sequence which encodes the heavy and/or light chain of the antibody molecule of any one of claims 1 to 21.

29. A cloning or expression vector containing the DNA sequence of claim 27.

10

30. An *E. coli* expression vector comprising the DNA sequence of claim 27.

31. The *E. coli* expression vector of claim 30 which is pTTOD(CDP791).

15

32. A host cell transformed with the vector of any one of claims 29 to 31.

33. A process for the production of the antibody molecule of any one of claims 1 to 21, comprising culturing the host cell of claim 32 and isolating the antibody molecule.

20

34. A process for the production of the antibody molecule of any one of claims 1 to 21, comprising culturing *E. coli* comprising an *E. coli* expression vector comprising the DNA sequence of any one of claims 29 to 31 and isolating the antibody molecule.

35. The process of claim 34 wherein the antibody molecule is targeted to the periplasm.

25

36. A therapeutic or diagnostic composition comprising the antibody molecule of any one of claims 1 to 21 or the compound of any one of claims 23 to 27.

37. The antibody molecule of any one of claims 1 to 21, having specificity for human

30

KDR, or the compound of any one of claims 23 to 27, for use in treating a pathology in which VEGF and/or KDR are implicated.

38. The antibody molecule of or compound claim 37, for use in treating inflammation, psoriasis, rheumatoid arthritis and tumour growth or metastasis.

39. Use of the antibody molecule of any one of claims 1 to 21, having specificity for 5 human KDR, or the compound of any one of claims 23 to 27 in the manufacture of a medicament for the treatment of a pathology in which VEGF and/or KDR are implicated.

40. The use of claim 39, wherein the pathology is inflammation, psoriasis, rheumatoid arthritis and tumour growth or metastasis.

10 41. The vector pTTOD(CDP791) as shown in Figure 8.

Figure 1: Sequence of CDRs of VR165**Heavy Chain**

H1 SYGMS (SEQ. ID. NO. 1)
H2 TITSGGSYTYYPDTVKKG (SEQ. ID. NO. 2)
H3 IGEDALDY (SEQ. ID. NO. 3)

Light Chain

L1 RASQDIAGSLN (SEQ. ID. NO. 4)
L2 ATSSLDS (SEQ. ID. NO. 5)
L3 LQYGSFPPT (SEQ. ID. NO. 6)

Figure 2: Protein sequence of mouse monoclonal antibody VR165 variable domains**Heavy Chain (SEQ. ID. NO. 7)**

VR165 EVQLVESGGDLVKPGGSLKLSGRAASGFTFSSYGMWSVRQTPDKRLQWVATITSGGSYTYYPDTVKKG

VR165 RFTISRDNAENTLYLQMSSLKSEDTAMYYCVRIGEDALDYWGQQGTSVTVSS

Light Chain (SEQ. ID. NO. 8)

VR165 DIQMTQSPSSLSASLGERVSLTCRASQDIAGSLNWLRQEPDGTIKRLIYATSSLDS

VR165 GVPKRFSGSRSGSDYSLTISSLESEDFVDYYCLQYGSFPPTFGGGSKLEIKR

Figure 3: Comparison of VR165 V-region to Chosen framework V-regions

Seq. Id. No. 9: VH3-7 GL V-region

VH3-7 GL	EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYWMSWVRQAPGKLEWVANIKQDGSEKYYVDSVKG
VR165	EVQLVESGGDLVKPGGSLKLSCAASGFTFSSYGMWSWVRQTPDKRLQWVATITSGGSYTYYPDTVKG

VH3-7 GL	RFTISRDNAKNSLYLQMNSLRAEDTAVYYCAR	WGQGTIVTVSS
VR165	RFTISRDNAENTLYLQMSSLKSEDTAMYYCVRIGEDALDYWGQGTSVTVSS	

Seq. Id. No.10: A30 GL V-region

A30 GL	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASSLQS
VR165	DIQMTQSPSSLSASLGERVSLTCRASQDIAGSLNWLRQEPDGTIKRLIYATSSLDS

A30 GL	GVPSRFSGSGSGTEFTLTISLQPEDFATYYCLQHNSYP	FGQGTIVKVEIKR
VR165	GVPKRFSGSRSGSDYSLTISSLESEDFVDYCLQYGSFPPTFGGGSKLEIKR	

Figure 4: Light Chain, including signal sequences

(Seq. Id. No. 13) ATG AAA AAG ACA GCT ATC GCA ATT GCA
TAC TTT TTC TGT CGA TAG CGT TAA CGT

(Seq. Id. No. 11) M K K T A I A I A>

(Seq. Id. No. 13) GTG GCC TTG GCT GGT TTC GCT ACC GTC GCG CAA GCT GAT ATC CAG ATG ACC CAG AGT
CAC CGG AAC CGA CCA AAG CGA TGG CAT CGC GTT CGA CTA TAG GTC TAC TGG GTC TCA

(Seq. Id. No. 11) V A L A G F A T V A Q A D I Q M T Q S>

(Seq. Id. No. 13) CCA AGC AGT CTC TCC GCC AGC GTC GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT
GGT TCG TCA GAG AGG CGG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA

(Seq. Id. No. 11) P S S L S A S V G D R V T I T C R A S>

(Seq. Id. No. 13) CAG GAC ATC GCG GGT AGC CTG AAC TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG
GTC CTG TAG CGC CCA TCG GAC TTG ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC

(Seq. Id. No. 11) Q D I A G S L N W L Q Q K P G K A I K>

(Seq. Id. No. 13) CGC CTC ATC TAT GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC
GCG GAG TAG ATA CGC TGC AGG TCG GAC CTA TCG CCA CAC GGT TTT GCA AAG TCA CGG

(Seq. Id. No. 11) R L I Y A T S S L D S G V P K R F S G>

(Seq. Id. No. 13) AGT CGC AGC GGT TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG CCG GAA GAT TTC
TCA GCG TCG CCA AGA CTG ATA TGG GAG GTG TAA AGC AGA GAG GTC GGC CTT CTA AAG

(Seq. Id. No. 11) S R S G S D Y T L T I S S L Q P E D F>

(Seq. Id. No. 13) GCC ACT TAC TAT TGT CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT
CGG TGA ATG ATA ACA GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA

(Seq. Id. No. 11) A T Y Y C L Q Y G S F P P T F G Q G T>

(Seq. Id. No. 13) AAA GTA GAA ATC AAA CGT ACG GTC GCG GCC CCA TCT GTC TTC ATC TTC CCG CCA TCT
TTT CAT CTT TAG TTT GCA TGC CAT CGC CGG GGT AGA CAG AAG TAG AAG GGC GGT AGA

(Seq. Id. No. 11) K V E I K R T V A A P S V F I F P P S>

(Seq. Id. No. 13) GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT
CTA CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC ACG GAC GAC TTA TTG AAG ATA

(Seq. Id. No. 11) D E Q L K S G T A S V V C L L N N F Y>

(Seq. Id. No. 13) CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC
GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG

(Seq. Id. No. 11) P R E A K V Q W K V D N A L Q S G N S>

(Seq. Id. No. 13) CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC
GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG TGG

(Seq. Id. No. 11) Q E S V T E Q D S K D S T Y S L S S T>

(Seq. Id. No. 13) CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC
GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG

(Seq. Id. No. 11) L T L S K A D Y E K H K V Y A C E V T>

(Seq. Id. No. 13) CAT CAG GGC CTG AGC TCA CCA GTC ACA AAA AGT TTT AAT AGA GGG GAG TGT TAA
GTA GTC CCG GAC TCG AGT GGT CAT TGT TTT TCA AAA TTA TCT CCC CTC ACA ATT

(Seq. Id. No. 11) H Q G L S S P V T K S F N R G E C *>

Figure 5: Heavy Chain, including signal sequences

(Seq. Id. No. 14) ATG AAG AAG ACT GCT ATA GCA ATT GCA GTG GCG CTA GCT GGT TTC GCC ACC GTG GCG CAA
 (Seq. Id. No. 12) TAC TTC TTC TGA CGA TAT CGT TAA CGT CAC CGC GAT CGA CCA AAG CGG TGG CAC CGC GTT
 M K K T A I A V A L A G F A T V A Q>

(Seq. Id. No. 14) GCT GAG GTT CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG CCT GGA GGG AGC CTG
 (Seq. Id. No. 12) CGA CTC CAA GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC GGA CCT CCC TCG GAC
 A E V Q L V E S G G G L V Q P G G S L>

(Seq. Id. No. 14) CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC GGT ATG TCC TGG GTG
 (Seq. Id. No. 12) GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG CCA TAC AGG ACC CAC
 R L S C A A S G F T F S S Y G M S W V>

(Seq. Id. No. 14) CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT ACG TCC GGA GGC AGC
 (Seq. Id. No. 12) GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA TGC AGG CCT CCG TCG
 R Q A P G K G L E W V A T I T S G G S>

(Seq. Id. No. 14) TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC CGG GAC AAT GCA
 (Seq. Id. No. 12) ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG GCC CTG TTA CGT
 Y T Y Y V D S V K G R F T I S R D N A>

(Seq. Id. No. 14) AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG GAC ACA GCA GTC TAT
 (Seq. Id. No. 12) TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC CTG TGT CGT CAG ATA
 K N T L Y L Q M N S L R A E D T A V Y>

(Seq. Id. No. 14) TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA CAG GGG ACC CTT GTG
 (Seq. Id. No. 12) ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT GTC CCC TGG GAA CAC
 Y C V R I G E D A L D Y W G Q G T L V>

(Seq. Id. No. 14) ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC
 (Seq. Id. No. 12) TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT AGC CAG AAG GGG GAC CGT GGG AGG AGG
 T V S S A S T K G P S V F P L A P S S>

(Seq. Id. No. 14) AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
 (Seq. Id. No. 12) TTC TCG TGG AGA CCC CCG TGT CGC CGG GAC CCG ACG GAC CAG TTC CTG ATG AAG GGG
 K S T S G G T A A L G C L V K D Y F P>

(Seq. Id. No. 14) GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC
 (Seq. Id. No. 12) CTT GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG TGG AAG
 E P V T V S W N S G A L T S G V H T F>

(Seq. Id. No. 14) CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC ACC AGC AGC GTG GTG ACC GTG CCC
 (Seq. Id. No. 12) GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG CAC CAC TGG CAC CGG
 P A V L Q S S G L Y S L S S V V T V P>

(Seq. Id. No. 14) TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC
 (Seq. Id. No. 12) AGG TCG TCG AAC CCG TGG GTC TGG ATG TAG AGC TTG CAC TTA GTG TTC GGG TCG TTG
 S S S L G T Q T Y I C N V N H K P S N>

(Seq. Id. No. 14) ACC AAG GTC GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC GCC
 (Seq. Id. No. 12) TGG TTC CAG CTG TTC TTT CAA CTC GGG TTT AGA ACA CTG TTT TGA GTG TGT ACG CGG
 T K V D K K V E P K S C D K T H T C A>

(Seq. Id. No. 14) GCG TGA
 CGC ACT
 (Seq. Id. No. 12) A *>

FIGURE 6

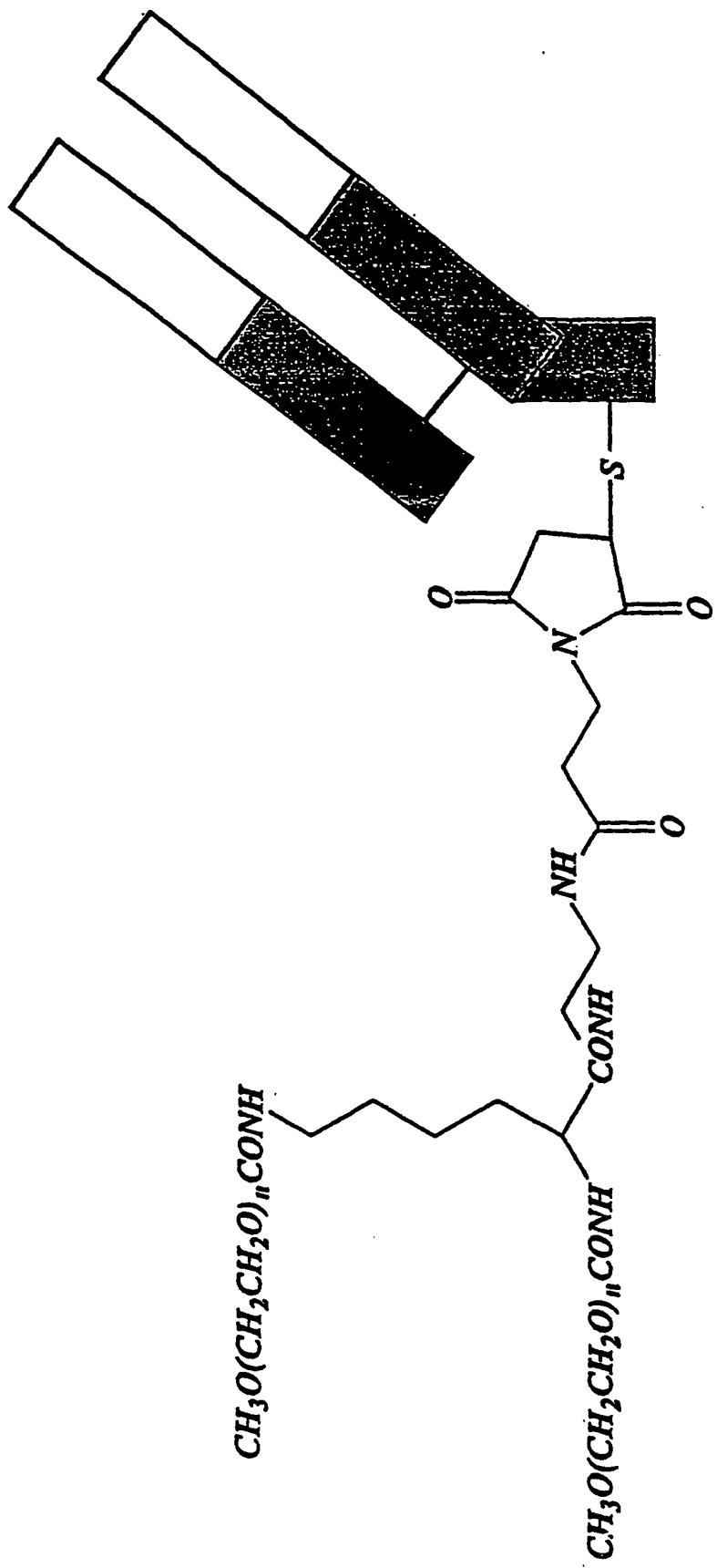


Figure 7:

gH3 (SEQ ID NO: 15)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEVVATIT
SGGSYTYYVDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDAL
DYWGQGTLVTVSS

gL3 (SEQ ID NO: 16)

DIQMTQSPSSLSASVGDRVITITCRASQDIAGSLNWLQQKPGKAIKRLIYATSSL
DSGVPKRFSGSRSGSDYTLTISSLQPEDFATYYCLQYGSFPPTFGQGTKVEIKR

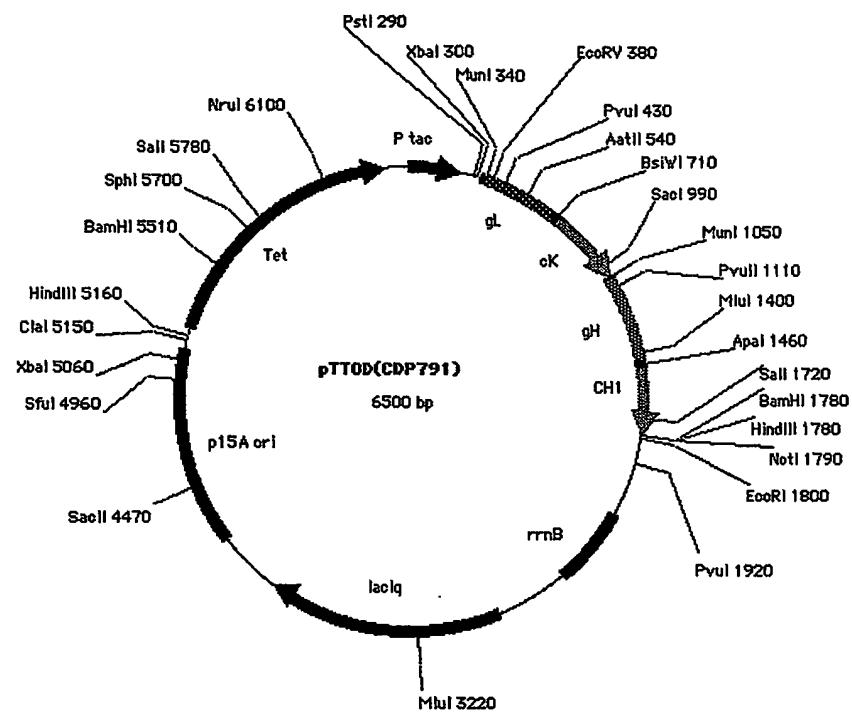
Figure 8: pTTOD(CDP791)

Figure 9: Protein sequence of the designed VH and VL grafts**Heavy Chain**

(Seq. Id. No. 17)

gH1 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYPDTVKG
gH1 RFTISRDNAKNSLYLQMNSLRAEDTAVYYCARIGEDALDYWGQGTLVTVSS

(Seq. Id. No. 18)

gH2 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYPDTVKG
gH2 RFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVTVSS

(Seq. Id. No. 19)

gH3 EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSYTYYVDSVKG
gH3 RFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVTVSS

Light Chain

(Seq. Id. No. 20)

gL1 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWLQQKPGKAPKRLIYATSSLDS
gL1 GVPKRFSGSGSGTEFTLTISLQPEDFATYYCLQYGSFPPTFGQGKVEIKR

(Seq. Id. No. 21)

gL2 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWLQQKPGKAPKRLIYATSSLDS
gL2 GVPKRFSGSRSGSDYTLTISLQPEDFATYYCLQYGSFPPTFGQGKVEIKR

(Seq. Id. No. 22)

gL3 DIQMTQSPSSLSASVGDRVTITCRASQDIAGSLNWLQQKPGKAIKRLIYATSSLDS
gL3 GVPKRFSGSRSGSDYTLTISLQPEDFATYYCLQYGSFPPTFGQGKVEIKR

Figure 10: Gene design**gH1 sequence (SEQ ID NO:23)**

HindIII 20 30 40 50 60
 GAATAAAAGC TTGCGGCCAC C ATG AAG ATG TGG TTA AAC TGG GTT TTC CTT GCC CTC ATT
 CTTATTTTCG AACGGCGGTG G TAC TTC TAC ACC AAT TTG ACC CAA AAG GAA CGG GAG TAA
 M K M W L N W V F L A L I>
 70 80 Pvull 100 110
 TTA AAA GGT GTC CAG TGT GAG GTG CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG
 AAT TTT CCA CAG GTC ACA CTC CAC GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC
 L K G V Q C E V Q L V E S G G G L V Q>
 120 130 140 150 160 170
 CCT GGA GGG AGC CTG CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC
 GGA CCT CCC TCG GAC GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG
 P G G S L R L S C A A S G F T F S S Y>
 180 190 200 210 220 230
 GGT ATG TCC TGG GTG CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT
 CCA TAC AGG ACC CAC GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA
 G M S W V R Q A P G K G L E W V A T I>
 BspEI 250 260 270 280
 ACG TCC GGA GGC AGC TAT ACA TAC TAC CCG GAC ACC GTC AAG GGC CGT TTC ACC ATT
 TGC AGG CCT CCG TCG ATA TGT ATG ATG GGC CTG TGG CAG TTC CCG GCA AAG TGG TAA
 T S G G S Y T Y Y P D T V K G R F T I>
 290 XmaI 300 310 320 330 340
 TCC CGG GAC AAT GCA AAG AAT AGC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG
 AGG GCC CTG TTA CGT TTC TTA TCG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC
 S R D N A K N S L Y L Q M N S L R A E>
 350 360 370 380 MluI 390 400
 GAC ACA GCA GTC TAT TAC TGT GCA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA
 CTG TGT CGT CAG ATA ATG ACA CGT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT
 D T A V Y Y C A R I G E D A L D Y W G>
 410 420 430 440 ApaI 450
 CAG GGG ACC CTT GTG ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA AGA AA
 GTC CCC TGG GAA CAC TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT TCT TT
 Q G T L V T V S S A S T K G P>

gL1 sequence (SEQ ID NO:24)

Sfui 20 30 40 50 60
 GGATGATTCG AAGCCGCCAC C ATG AGG ACC CCT GCT CAG ATT CTT GGC TTC TTG TTG CTC
 CCTACTAACG TTGGCGGTG G TAC TCC TGG GGA CGA GTC TAA GAA CCG AAG AAC AAC GAG
 M R T P A Q I L G F L L L>
 70 80 EcorV 90 100 110
 TTG TTT CCA GGT ACC AGA TGT GAT ATC CAG ATG ACC CAG AGT CCA AGC AGT CTC TCC
 AAC AAA GGT CCA TGG TCT ACA CTA TAG GTC TAC TGG GTC TCA GGT TCG TCA GAG AGG
 L F P G T R C D I Q M T Q S P S S L S>
 120 130 140 150 160 170
 GCC AGC GTC GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT CAG GAC ATC GCG GGT
 CCG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA GTC CTG TAG CGC CCA
 A S V G D R V T I T C R A S Q D I A G>
 180 190 200 210 220 230
 AGC CTG AAC TGG TAT CAG CAA AAA CCG GGC AAA GCC CCC AAG CGC CTC ATC TAT GCG
 TCG GAC TTG ACC ATA GTC GTT TTT GGC CCG TTT CGG GGG TTC GCG GAG TAG ATA CGC
 S L N W Y Q Q K P G K A P K R L I Y A>
 AatII 240 250 260 270 280
 ACG TCC AGC CTG GAT AGC GGT GTG CCA TCT CGT TTC AGT GGC AGT GGC AGC GGT ACT
 TGC AGG TCG GAC CTA TCG CCA CAC GGT AGA GCA AAG TCA CGG TCA CCG TCG CCA TGA
 T S S L D S G V P S R F S G S G S G T>
 290 300 BsmBI 320 330 340
 GAA TTT ACC CTC ACA ATT TCG TCT CTC CAG CGG GAA GAT TTC GGC ACT TAC TAT TGT
 CTT AAA TGG GAG TGT TAA AGC AGA GAG GTC GGC CTT CTA AAG CGG TGA ATG ATA ACA
 E F T L T I S S L Q P E D F A T Y Y C>

350 360 370 380 390 400
CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT AAA GTA GAA ATC AAA
GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA TTT CAT CTT TAG TTT
L Q Y G S F P P T F G Q G T K V E I K>

BsiWI 410
CGT ACG GC GTGC
GCA TGC CG CACG
R T>

Figure 11: Oligonucleotides for gene assembly**gL1 F1 (SEQ ID NO:25)**

GGATGATTGAAAGCCGCCAC

gL1 F2 (SEQ ID NO:26)TCCAGGTACAGATGTGATATCCAGATGACCCAGAGTCCAAGCAGTCTCTCCGCCAGCGTAGGCGATCGTG
TGACTATTACCTGTC**gL1 F3 (SEQ ID NO:27)**CAAAAACCGGGCAAGCCCCAAGCGCCTCATCTATGCGACGTCCAGCCTGGATAGCGGTGCCCCATCTCG
TTTCAGTGGCAGTGGC**gL1 F4 (SEQ ID NO:28)**AGATTCGCCACTTACTATTGCTGCAATATGGCAGCTCCCTCCGACCTTCGGTCAGGGCACTAAAGTAGA
AATCAAACGTACGGCGTGC**gL1 R1 (SEQ ID NO:29)**

GCACGCCGTACGTTGATTTC

gL1 R2 (SEQ ID NO:30)GACAATAGTAAGTGGCGAAATCTCCGGCTGGAGAGAGACGAAATTGTGAGGGTAAATTCAGTACCGCTGCCA
CTGCCACTGAAACGAG**gL1 R3 (SEQ ID NO:31)**GGGGCTTGGCCGGTTTGCTGATACCAGTTCAGGCTACCCGCGATGTCCTGACTGGCACGACAGGTAAT
AGTCACACGATC**gL1 R4 (SEQ ID NO:32)**GATATCACATCTGGTACCTGGAAACAAGAGCAACAAGAAGCCAAGAATCTGAGCAGGGTCCTCATGGTGG
CGGCTTCGAATCATCC**gH1 F1 (SEQ ID NO:33)**

GAATAAAAGCTTGGCCGCCACC

gH1 F2 (SEQ ID NO:34)TCCAGTGTGAGGTGAGCTGGAGTCTGGAGGGGGCTTGTCCAGCCTGGAGGGAGCCTGCGTCTCTC
TTGTGCAAGCGGCTTCAC**gH1 F3 (SEQ ID NO:35)**AGTGGGTGGCAACCATTACGTCCGGAGGCAGCTATACTACATACTACCCGGACACCGTCAAGGGCCGTTTCA
ATTTCCCGGGACAATGCAA**gH1 F4 (SEQ ID NO:36)**CTATTACTGTGCACGGATCGGCGAAGACGCGTTGGACTACTGGGGACAGGGGACCCCTGTGACAGTCTCCT
CTGCTTCTACAAGGGCCCAAGAAA**gH1 R1 (SEQ ID NO:37)**

TTCTTGGGCCCTTGTAGAAG

gH1 R2 (SEQ ID NO:38)CCGATCCGTGCACAGTAATAGACTGCTGTGTCCTCTGCGCGAGAGAGTCATCTGGAGGTAAAGGCTATT
CTTTCGATTGTCCCGGGAAATGG**gH1 R3 (SEQ ID NO:39)**ACGTAATGGTTGCCACCCACTCCAGGCCCTCCAGGTGCCTGCCGCACCCAGGACATACCGTAAGAGGAA
AAGGTGAAGCCGCTGCTGCACA**gH1 R4 (SEQ ID NO:40)**CAGCTGCACCTCACACTGGACACCTTTAAATGAGGGCAAGGAAAACCCAGTTAACCATCTTCATGGT
GGCGGCAAGCTTTATTG

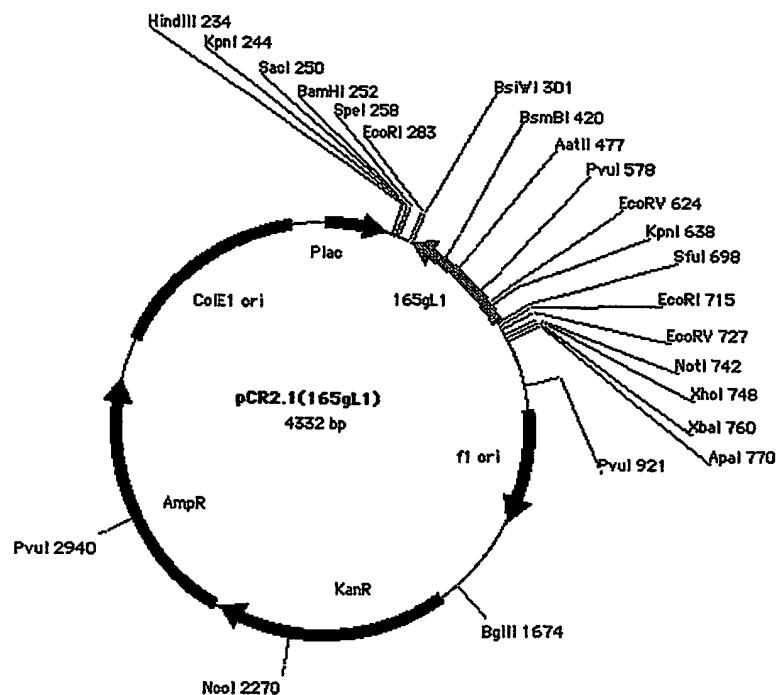
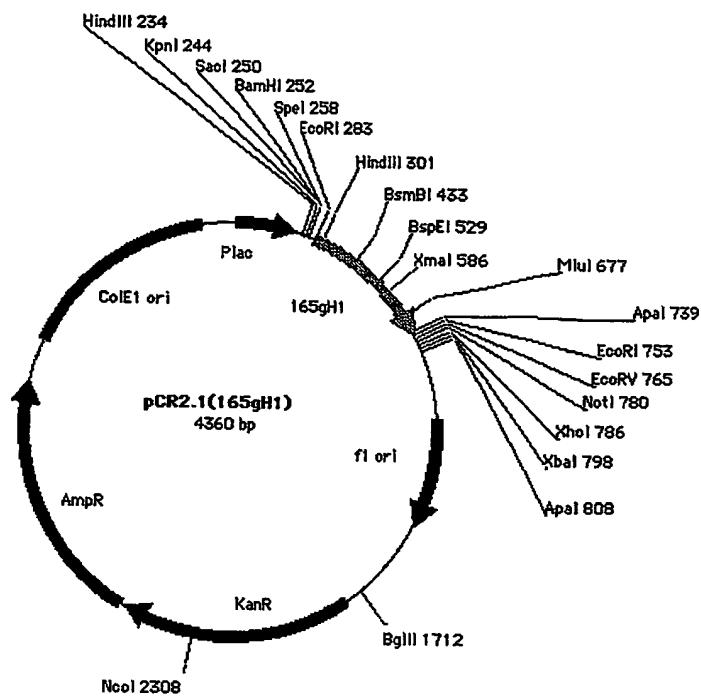
Figure 12: Plasmid pCR2.1(gH1) and pCR2.1(gL1)

Figure 13: Oligonucleotide cassettes for construction of gH2, gH3, gL2, gL3**gH2 cassette (SEQ ID NO: 41)**

XmaI 10 20 30 40 50
TCC CCG GAC AAT GCA AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG
AGG GCC CTG TTA CGT TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC
S R D N A K N T L Y L Q M N S L R A E>
60 70 80 90 MluI
GAC ACA GCA GTC TAT TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG
CTG TGT CGT CAG ATA ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC
D T A V Y Y C V R I G E D A L>

gH3 cassette (SEQ ID NO:42)

BspEI 10 20 30 40 50
TCC GGA GGC AGC TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC
AGG CCT CCG TCG ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG
S G G S Y T Y Y V D S V K G R F T I S>
XmaI
CGG GAC
GCC CTG
R D>

gL2 cassette (SEQ ID NO:43)

AatII 10 20 30 40 50
GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC AGT CGC AGC GGT
CGC TGC AGG TCG GAC CTA TCG CCA CAC GGT TTT GCA AAG TCA CCG TCA GCG TCG CCA
A T S S L D S G V P K R F S G S R S G>
60 70 80 BsmBI 90
TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG
AGA CTG ATA TGG GAG TGT TAA AGC AGA GAG GTC
S D Y T L T I S S L Q>

gL3 cassette (SEQ ID NO:44)

PvuI 10 20 30 40 50
GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT CAG GAC ATC GCG GGT AGC CTG AAC
CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA GTC CTG TAG CGC CCA TCG GAC TTG
G D R V T I T C R A S Q D I A G S L N>
60 70 80 90 100 AatII
TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG CGC CTC ATC TAT GCG ACG TCC
ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC GCG GAG TAG ATA CGC TGC AGG
W L Q Q K P G K A I K R L I Y A T S>

Underlined residues represent changed amino acids compared to the parent sequence.

Figure 14: Oligonucleotides pairs for gH2, gH3, gL2, gL3 construction**gH2T (SEQ ID NO:45)**

CCGGGACAATGCAAAGAATACCCTTACCTCCAGATGAACCTCTCCGCGCAGAGGACACAGCAGT
CTATTACTGTGTACGGATCGGCGAAGA

gH2B (SEQ ID NO:46)

CGCGTCTCGCCGATCCGTACACAGTAATAGACTGCTGTGCCTCTGCGCGGAGAGAGTTCATCTG
GAGGTAAAGGGTATTCTTGCATTGTC

gH3T (SEQ ID NO:47)

CCGGAGGGCAGCTATACTACGTGGACAGCGTCAAGGGCCGTTTACCCATTTC

gH3B (SEQ ID NO:48)

CCGGGAAATGGTGAAACGGCCCTTGACGCTGTCCACGTAGTATGTATAGCTGCCT

gL2T (SEQ ID NO:49)

CCAGCCTGGATAGCGGTGTGCCAAAACGTTTCAGTGGCAGTCGCAAGCGTTCTGACTATAACCTC
ACAATTTCGTCTCT

gL2B (SEQ ID NO:50)

CTGGAGAGACGAAATTGTGAGGGTATAGTCAGAACCGCTGCGACTGCCACTGAAACGTTGGCA
CACCGCTATCCAGGCTGGACGT

gL3T (SEQ ID NO:51)

CGTGTGACTATTACCTGTGTGCCAGTCAGGACATCGCGGGTAGCCTGAACGGTTGCAGCAAAA
ACCGGGCAAAGCCATCAAGCGCCTCATCTATGCGACGT

gH3B (SEQ ID NO:52)

CGCATAGATGAGGCGCTTGTGGCTTGCCGGTTTGCTGCAACCAGTTCAAGGCTACCCGCGAT
GTCCTGACTGGCACGACAGGTAATAGTCACACGAT

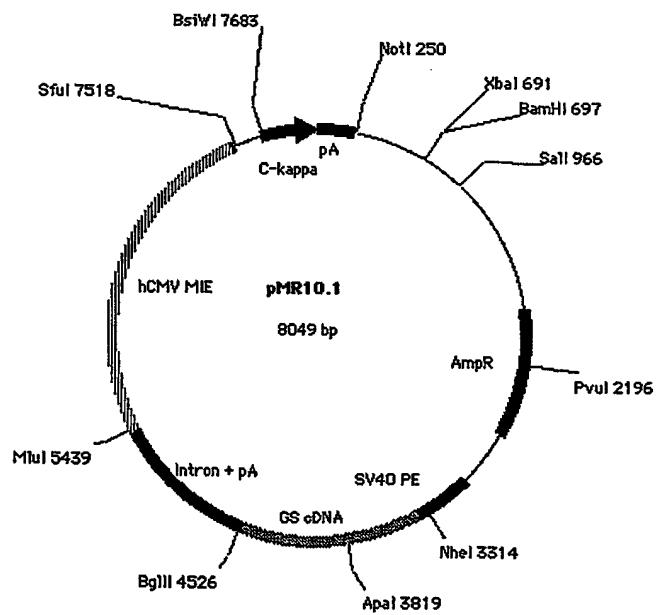
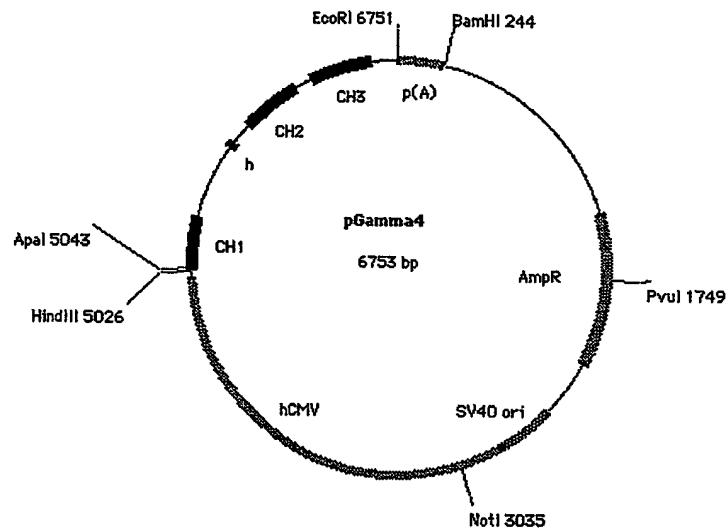
Figure 15: CHO expression plasmids

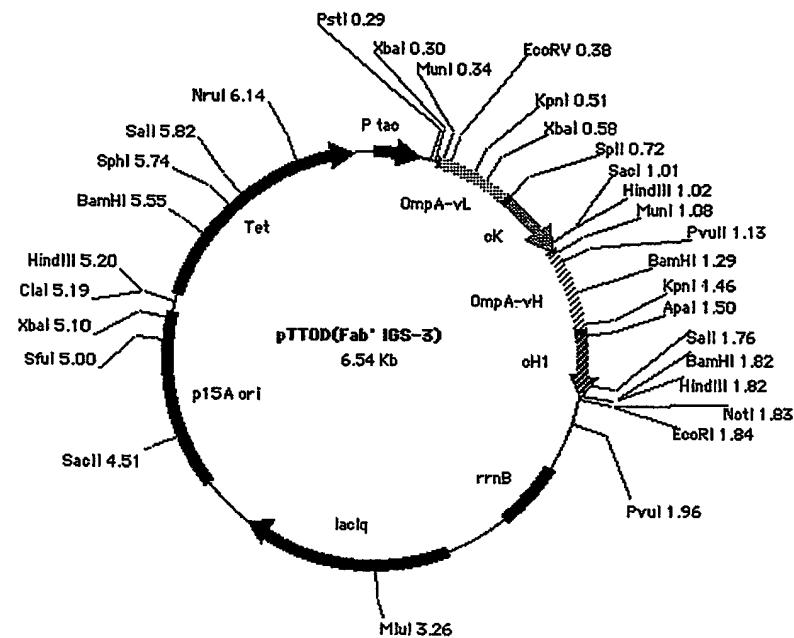
Figure 16: pTTOD(Fab' IGS-3) map

Figure 17: IGS Sequences

(SEQ ID NO:53)

IGS-1; Intergenic space = -1G, AGC, TCA, CCA, GTA, ACA, AAA, AGT, TTT, AAT, AGA, GGA, GAG, TGT, TAATG, AAG, ACT, GCT, ATA, GCA, ATT, G

S	S	P	V	T	K	S	F	N	R	G	E	C	*	M	K	K	T	A	I	A	I
Start of c-Kappa sequence ->																					

(SEQ ID NO:54)

IGS-2; Intergenic space = +1G, AGC, TCA, CCA, GTA, ACA, AAA, AGT, TTT, AAT, AGA, GGG, GAG, TGT, TAA AATG, AAG, ACT, GCT, ATA, GCA, ATT, G

S	S	P	V	T	K	S	F	N	R	G	E	C	*	M	K	K	T	A	I	A	I
Start of OmpA sequence ->																					

(SEQ ID NO:55)

IGS-3; Intergenic space = +13G, AGC, TCA, CCA, GTA, ACA, AAA, AGC, TTT, AAT, AGA, GGA, GAG, TGT, TGA GGGGAAAAAAATG, AAG, AAA, ACT, GCT, ATA, GCA, ATT, G

S	S	P	V	T	K	S	F	N	R	G	E	C	*	M	K	K	T	A	I	A	I
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

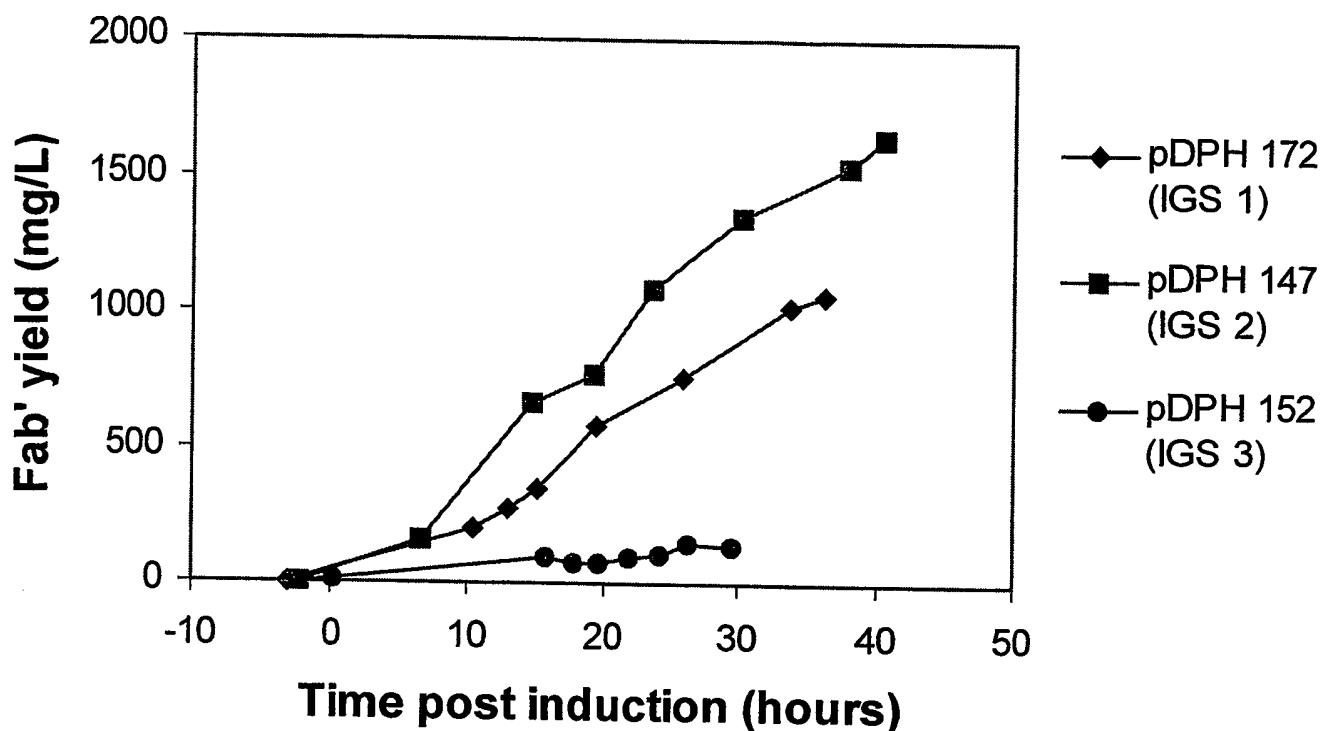
Figure 18: Fermentation of Fab'

Figure 19: Coding and flanking sequence of CDP791 Fab' (SEQ ID NO:56)

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TTAAGAGTAC AAACTGTCGA ATAGTAGCTG ACGTGCCACG TGGTTACGAA GACCGCAGTC CGTCGGTAGC						
80	90	100	110	120	130	140
GAAGCTGTGG TATGGCTGTG CAGGTCGTAATCACTGCAT AATTCTGTC GCTCAAGGCG CACTCCCGTT						
CTTCGACACC ATACCGACAC GTCCAGCATT TAGTGCAGTA TTAAGCACAG CGAGTTCGCG GTGAGGGCAA						
150	160	170	180	190	200	210
CTGGATAATG TTTTTTSGCG CGACATCATCA ACGGTTCTGG CAAATATTCT GAAATGAGCT GTTGACAATT						
GACCTATTAC AAAAACCGCG GCTGTAGTAT TGCCAAGACC GTTTATAAGA CTTTACTCGA CAACTGTTAA						
220	230	240	250	260	270	280
AATCATCGGC TCGTATAATG TGTTGAAATTG TGAGCGGATA ACAATTTCAC ACAGGAAACA GCGATGAGCT						
TTAGTAGCCG AGCATATTAC ACACCTAAC ACTCGCCTAT TGTTAAAGTG TGTCCTTGT CGCTACTCGA						
290	300	310	320	330	340	
TGGCTGCAGG TCGAGTTCTA GATAACGAGG CGTAAAAA ATG AAA AAG ACA GCT ATC GCA ATT GCA						
ACCGACGTCC AGCTCAAGAT CTATTGCTCC GCATTTT TAC TTT TTC TGT CGA TAG CGT TAA CGT						
				M	K	K
				T	A	I
				A	I	A
						A>
350	360	370	380	390	400	
GTG GCC TTG GCT GGT TTC GCT ACC GTA GCG CAA GCT GAT ATC CAG ATG ACC CAG AGT						
CAC CGG AAC CGA CCA AAG CGA TGG CAT CGC GTT CGA CTA TAG GTC TAC TGG GTC TCA						
V	A	L	A	G	F	A>
				A	T	V
				Q	A	D
					I	Q
					M	T
					Q	S>
410	420	430	440	450		
CCA AGC AGT CTC TCC GCC AGC GTC GGC GAT CGT GTG ACT ATT ACC TGT CGT GCC AGT						
GGT TCG TCA GAG AGG CGG TCG CAT CCG CTA GCA CAC TGA TAA TGG ACA GCA CGG TCA						
P	S	S	L	S	A	S>
				V	G	D
				D	R	R
				V	T	I
					T	C
					R	R
					A	A
					S>	
460	470	480	490	500	510	
CAG GAC ATC GCG GGT AGC CTG AAC TGG TTG CAG CAA AAA CCG GGC AAA GCC ATC AAG						
GTC CTG TAG CGC CCA TCG GAC TTG ACC AAC GTC GTT TTT GGC CCG TTT CGG TAG TTC						
Q	D	I	A	G	S	L>
				L	N	W
				Q	Q	K
					K	P
					P	G
					G	K
					A	I
						K>
520	530	540	550	560	570	
CGC CTC ATC TAT GCG ACG TCC AGC CTG GAT AGC GGT GTG CCA AAA CGT TTC AGT GGC						
GCG GAG TAG ATA CGC TGC AGG TCG GAC CTA TCG CCA AAC GGT TTT GCA AAG TCA CCG						
R	L	I	Y	A	T	S>
				S	S	L
				L	D	S
				Q	S	G
					V	P
					K	R
					F	S
					S>	G>
580	590	600	610	620	630	
AGT CGC AGC GGT TCT GAC TAT ACC CTC ACA ATT TCG TCT CTC CAG CCG GAA GAT TTC						
TCA GCG TCG CCA AGA CTG ATA TGG GAG TGT TAA AGC AGA GAG GTC GGC CTT CTA AAG						
S	R	S	G	S	D	Y>
					T	T
					L	T
					I	S
					S	S
					L	Q
					P	E
					D	F>
640	650	660	670	680		
GCC ACT TAC TAT TGT CTG CAA TAT GGC AGC TTC CCT CCG ACC TTC GGT CAG GGC ACT						
CGG TGA ATG ATA ACA GAC GTT ATA CCG TCG AAG GGA GGC TGG AAG CCA GTC CCG TGA						
A	T	Y	Y	C	L	Q>
					Q	Y
					G	S
					S	F
					P	P
					T	T
					F	G
					Q	Q
					G	G
					T>	
690	700	710	720	730	740	
AAA GTA GAA ATC AAA CGT ACG GTA GCG GCC CCA TCT GTC TTC ATC TTC CCG CCA TCT						
TTT CAT CTT TAG TTT GCA TGC CAT CGC CGG GGT AGA CAG AAG TAG AAG GGC GGT AGA						
K	V	E	I	K	R	T>
					T	V
					A	A
					P	S
					S	V
					F	F
					I	F
					F	P
					P	P
					S>	
750	760	770	780	790	800	
GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT						
CTA CTC GTC AAC TTT AGA CCT TGA CGG AGA CAA CAC AGC GAC GAC TTA TTG AAG ATA						
D	E	Q	L	K	S	Y>
					G	T
					T	A
					S	V
					V	V
					C	C
					L	L
					N	N
					F	F
					S>	
810	820	830	840	850		
CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC						
GGG TCT CTC CGG TTT CAT GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG						
P	R	E	A	K	V	Y>
					Q	W
					W	K
					K	D
					S	S
					D	N
					N	A
					A	L
					Q	S
					S	G
					G	N
					S>	
860	870	880	890	900	910	
CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC						
GTC CTC TCA CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG TGG						
Q	E	S	V	T	E	Y>
					Q	Q
					D	S
					S	T
					Y	Y
					S	S
					L	S
					S	S
					T>	

920 930 940 950 960 970
 CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC
 GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG CTT CAG TGG
 L T L S K A D Y E K H K V Y A C E V T>
 980 990 1000 1010 1020 1030
 CAT CAG GGC CTG AGC TCA CCA GTA ACA AAA AGT TTT AAT AGA GGG GAG TGT TAA A ATG
 GTA GTC CCG GAC TCG AGT GGT CAT TGT TTT TCA AAA TTA TCT CCC CTC ACA ATT T TAC
 H Q G L S S P V T K S F N R G E C *>
 M>
 1040 1050 1060 1070 1080
 AAG AAG ACT GCT ATA GCA ATT GCA GTG GCG CTA GCT GGT TTC GCC ACC GTG GCG CAA
 TTC TTC TGA CGA TAT CGT TAA CGT CAC CGC GAT CGA CCA AAG CGG TGG CAC CGC GTT
 K K T A I A I A V A L A G F A T V A Q>
 1090 1100 1110 1120 1130 1140
 GCT GAG GTT CAG CTG GTC GAG TCT GGA GGC GGG CTT GTC CAG CCT GGA GGG AGC CTG
 CGA CTC CAA GTC GAC CAG CTC AGA CCT CCG CCC GAA CAG GTC GGA CCT CCC TCG GAC
 A E V Q L V E S G G L V Q P G G S L>
 1150 1160 1170 1180 1190 1200
 CGT CTC TCT TGT GCA GCA AGC GGC TTC ACC TTT TCC TCT TAC GGT ATG TCC TGG GTG
 GCA GAG AGA ACA CGT CGT TCG CCG AAG TGG AAA AGG AGA ATG CCA TAC AGG ACC CAC
 R L S C A A S G F T F S S Y G M S W V>
 1210 1220 1230 1240 1250
 CGG CAG GCA CCT GGG AAG GGC CTG GAG TGG GTG GCA ACC ATT ACG TCC GGA GGC AGC
 GCC GTC CGT GGA CCC TTC CCG GAC CTC ACC CAC CGT TGG TAA TGC AGG CCT CCG TCG
 R Q A P G K G L E W V A T I T S G G S>
 1260 1270 1280 1290 1300 1310
 TAT ACA TAC TAC GTG GAC AGC GTC AAG GGC CGT TTC ACC ATT TCC CGG GAC AAT GCA
 ATA TGT ATG ATG CAC CTG TCG CAG TTC CCG GCA AAG TGG TAA AGG GCC CTG TTA CGT
 Y T Y Y V D S V K G R F T I S R D N A>
 1320 1330 1340 1350 1360 1370
 AAG AAT ACC CTT TAC CTC CAG ATG AAC TCT CTC CGC GCA GAG GAC ACA GCA GTC TAT
 TTC TTA TGG GAA ATG GAG GTC TAC TTG AGA GAG GCG CGT CTC CTG TGT CGT CAG ATA
 K N T L Y L Q M N S L R A E D T A V Y>
 1380 1390 1400 1410 1420
 TAC TGT GTA CGG ATC GGC GAA GAC GCG TTG GAC TAC TGG GGA CAG GGG ACC CCT GTG
 ATG ACA CAT GCC TAG CCG CTT CTG CGC AAC CTG ATG ACC CCT GTC CCC TGG GAA CAC
 Y C V R I G E D A L D Y W G Q G T L V>
 1430 1440 1450 1460 1470 1480
 ACA GTC TCC TCT GCT TCT ACA AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC
 TGT CAG AGG AGA CGA AGA TGT TTC CCG GGT AGC CAG AAG GGG GAC CGT GGG AGG AGG
 T V S S A S T K G P S V F P L A P S S>
 1490 1500 1510 1520 1530 1540
 AAG ACC ACC TCT GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC
 TTC TCG TGG AGA CCC CCG TGT CGC CGG GAC CGG ACG GAC CAG TTC CTG ATG AAG GGG
 K S T S G G T A A L G C L V K D Y F P>
 1550 1560 1570 1580 1590 1600
 GAA CCG GTG AGC GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC
 CTT GGC CAC TGC CAC AGC ACC TTG AGT CCG CGG GAC TGG TCG CCG CAC GTG TGG AAG
 E P V T V S W N S G A L T S G V H T F>
 1610 1620 1630 1640 1650
 CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC
 GGC CGA CAG GAT GTC AGG AGT CCT GAG ATG AGG GAG TCG TCG CAC CAC TGG CAC GGG
 P A V L Q S S G L Y S L S S V V T V P>
 1660 1670 1680 1690 1700 1710
 TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC
 AGG TCG TCG AAC CCG TGG GTC TGG ATG TAG AGC TTG CAC TTA GTG TTC GGG TCG TTG
 S S S L G T Q T Y I C N V N H K P S N>
 1720 1730 1740 1750 1760 1770
 ACC AAG GTC GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC GCC
 TGG TTC CAG CTG TTC TTT CAA CTC GGG TTT AGA ACA ACT CGT TTT TGA GTG TGT ACG CGG
 T K V D K K V E P K S C D K T H T C A>

1780 1790 1800 1810 1820 1830 1840
GCG TGA TGA GGATCCAAGC TTGCGGCCGC GAATTCACTG GCCGTCGTT TACAACGTCG TGACTGGGAA
CGC ACT ACT CCTAGGTTCG AACGCCGGCG CTTAAGTGAC CGGCAGCAAA ATGTTGCAGC ACTGACCCCTT
A * >

1850 1860 1870 1880 1890 1900 1910
AACCCCTGGCG TTACCCAACT TAATCGCCTT GCAGCACATC CCCCTTCGC CAGCTCGCGT AATAGCGAAG
TTGGGACCGC AATGGGTTGA ATTAGCGGAA CGTCGTGTAG GGGGAAAGCG GTCGAGCGCA TTATCGCTTC

1920 1930 1940 1950 1960 1970 1980
AGGCCCGCAC CGATGCCCT TCCCAACAGT TGCGCAGCCT GAATGGCGAA TGGCGCCTGA TGCGGTATTT
TCCGGGCGTG GCTAGCGGGA AGGGTTGTCA ACGCGTCGGA CTTACCGCTT ACCGCGGACT ACGCCATAAA

1990 2000
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AGAGGAATGC GTAGACACGC

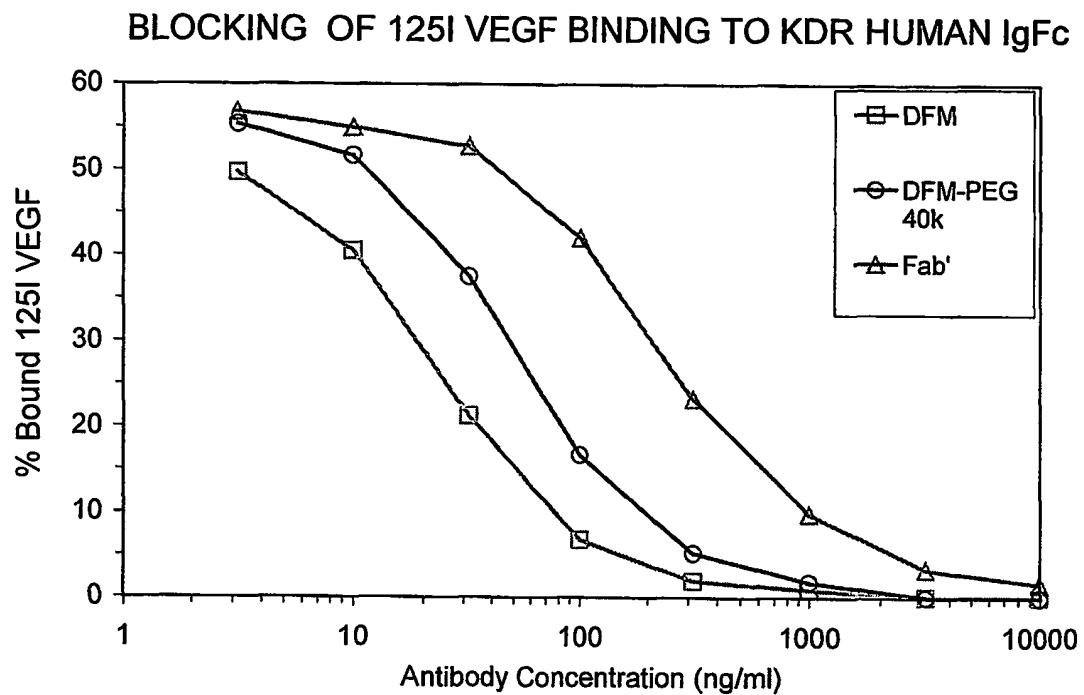
Figure 20: Radioimmunoassay Results

Figure 21: Protein sequence of gH3-grafted heavy chain

Heavy Chain antibody sequence (SEQ ID NO:57)

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVATITSGGSY
TYYVDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCVRIGEDALDYWGQGTLVT
VSSASTKGPSVFPLAPCSRSTSESTAAAGCLVKDYFPEPVTVSWNSGALTSGVHTFP
AVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVVDHKPSNTKVDKRVESKYGPPCPSCPA
PEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAK
TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDG
SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGK*